Glucose intolerance and steroid sex hormones in the aetiology of peripheral arterial disease

Jacqueline Frances Price

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Declaration

a) This thesis was composed by myself, Jacqueline F Price.

b) The case control study was designed, performed, analysed and written by myself, with the exception of the multiple linear regression modelling used to determine the relationship between smoking status and serum insulin. This was performed at my request by Dr AJ Lee (statistician, Wolfson Unit for Prevention of Peripheral Vascular Diseases, University of Edinburgh).

c) The Edinburgh Artery Study was designed and conducted by Professor FGR Fowkes (Professor of Epidemiology, University of Edinburgh). For the work presented here on the relationship between cardiovascular risk factors, diabetes and peripheral arterial disease, I was responsible for identifying and investigating the area of interest, formulating and directing the statistical analysis, interpreting the results and writing the scientific paper. The statistical analysis was performed at my request by Dr AJ Lee. Dr AS McGregor (research fellow, Wolfson Unit for Prevention of Peripheral Vascular Diseases) also contributed to the writing of the scientific paper under my direct supervision.

d) This thesis has not been submitted for any other degree, diploma or professional qualification.

Jacqueline F Price Date: 26.09.01

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ABSTRACT OF THESIS

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Name of Candidate JACQUELINE FRANCES PRICE

Address .....

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The aetiology of peripheral arterial disease is likely to be multifactorial. Aside from conventional risk factors, 'endocrine' risk factors such as glucose intolerance and steroid sex hormones may be important. These factors were investigated in the Edinburgh Artery Study, a large prospective study on the epidemiology of peripheral arterial disease.

The prevalence of peripheral arterial disease is known to be higher in subjects with diabetes mellitus or impaired glucose tolerance compared with non-diabetic subjects. The objective of study 1 was to determine whether this could be explained by differing levels of 'traditional' risk factors, such as smoking, hypertension, dyslipidaemia and obesity. **Methods**: 1,592 men and women aged 55-74 years were selected at random from the age-sex registers of 11 general practices throughout Edinburgh, Scotland. Subjects underwent a comprehensive medical examination, including assessment for peripheral arterial disease (positive intermittent claudication questionnaire or major asymptomatic disease on non-invasive testing), a glucose tolerance test and measurement of cardiovascular risk factors (including smoking, blood pressure, body mass index and serum lipids and lipoproteins). **Results**: 288 subjects (18.7%) were found to have diabetes or impaired glucose tolerance (IGT). The prevalence of peripheral arterial disease was greater in subjects with diabetes or IGT (20.6%) compared to those with normal glucose tolerance (12.5%) (age and sex-adjusted OR 1.45; 95% CI 1.03, 2.04). In subjects with diabetes or IGT, mean levels of smoking, systolic blood pressure and serum triglycerides were significantly higher in subjects with peripheral arterial disease than in those without disease ($p \leq 0.05$). In general, levels of cardiovascular risk factors were higher in subjects with diabetes or IGT compared with normal glucose tolerant subjects; this included systolic blood pressure and triglycerides, but not smoking. In multivariate analysis, subjects with diabetes or IGT no longer had a significantly higher risk of peripheral arterial disease after adjusting separately for systolic blood pressure (OR 1.22, 95% CI 0.85, 1.73) or serum triglycerides (OR 1.26, 95% CI 0.89, 1.79). Simultaneous adjustment for both risk factors reduced the odds of disease further to 1.11 (95% CI 0.78, 1.58). **Conclusions**: Raised levels of serum triglycerides and systolic blood pressure in subjects with diabetes or IGT may explain a major portion of their increased risk of peripheral arterial disease.

The objectives of study 2 were to determine whether, in non-diabetic men and women from the general population, there was an association between peripheral arterial disease and (i) plasma insulin levels or (ii) endogenous steroid sex hormones. **Methods**: 83 cases with peripheral arterial disease and 88 age and sex matched controls were selected from non-diabetic participants in the Edinburgh Artery Study. Venous blood samples were taken for measurement of steroid sex hormones and for plasma insulin, both in the fasting state and one hour after a standard oral glucose tolerance test. **Results**: Compared with controls, cases had higher mean plasma insulin levels one hour after the oral glucose load (73.6 mU/l vs 59.8 mU/l; $p \leq 0.05$). The relationship between one-hour insulin and disease was independent of blood pressure (OR 2.04; 95% CI 1.11,3.74; $p \leq 0.05$) and partially independent of serum low and high density lipoprotein cholesterol and triglycerides (OR 1.86; 95% CI 0.99,3.48; $p \leq 0.1$). However, when smoking was added to the multivariate model, the relationship between insulin and disease diminished (odds ratio 1.64; 95% CI 0.83,3.23; $p > 0.1$), consistent with raised mean insulin levels in smokers. Mean plasma oestrone levels were slightly higher in male cases than controls (101.9 pmol/l vs 92.1 pmol/l; $p = 0.09$), but this association lost significance after multivariate adjustment for age and body mass index. Mean levels of plasma total and free testosterone, oestradiol and sex hormone-binding globulin were not significantly different in cases compared with controls in either sex ($p > 0.1$). **Conclusions**: In the non-diabetic general population, peripheral arterial disease was associated with non-fasting hyperinsulinaemia, independent of blood pressure, serum lipoproteins and triglycerides. Some of the association may have been mediated by a relationship between hyperinsulinaemia and smoking. Peripheral arterial disease was not associated with altered levels of individual endogenous steroid sex hormones in either men or postmenopausal women. The implications of these findings are discussed in the context of previous research.

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ABPI	ankle brachial pressure index
BMI	body mass index
CI	confidence interval
HDL	high density lipoprotein
IC	intermittent claudication
IGT	impaired glucose tolerance
IHD	ischaemic heart disease
IMT	intimal medial thickness
LDL	low density lipoprotein
LRC	Lipid Research Clinic
NGT	normal glucose tolerance
NS	not significant
OGTT	oral glucose tolerance test
OPCS	Office of Population Censuses and Surveys
PAD	peripheral arterial disease
SD	standard deviation
SE	standard error
USA	United States America
USS	ultrasound scan
UK	United Kingdom
WHO	World Health Organization
WHR	waist hip ratio

Chapter I

Introduction

Epidemiology of peripheral arterial disease, aims and objectives

1.1 Definition and classification of peripheral arterial disease

In the strictest sense, 'peripheral arterial disease' refers to all arterial pathology occurring outwith the coronary arteries, including the arms, carotid arteries, cerebral arteries and renal arteries as well as the aorta and arteries of the lower limbs. However, the term is frequently used to refer to disease of the lower limb arteries alone. Such disease includes conditions such as inflammatory arteritis, vasospastic disorders and medial calcification, but by far the most common cause of peripheral arterial disease is atherosclerosis.

For the purposes of this thesis, as in general parlance, the term 'peripheral arterial disease' is used to refer to atherosclerotic disease of the lower limbs. Indeed, in most epidemiological studies, causes of peripheral arterial disease other than atherosclerosis cannot be easily differentiated; it is generally accepted that inclusion of these few cases will have a minimal effect on the overall results. Also consistent with usual terminology, 'cardiovascular disease' is used to include both peripheral arterial disease and coronary

artery disease (generally myocardial infarction and angina), a condition for which the most common underlying pathology is also atherosclerosis.

Atherosclerotic disease of the lower limbs ranges from mild asymptomatic disease to severe limb-threatening ischaemia. Intermittent claudication is the prime symptom, characterised by pain in the muscles of the leg when walking which is relieved by rest and provoked by an inadequate arterial blood flow during exercise. At the more severe end of the spectrum of disease is critical limb ischaemia, a term used to describe those patients whose arterial disease has led to rest pain in the foot or tissue necrosis (ulceration or gangrene). More formal definitions of critical limb ischaemia have required the presence of a reduced ankle systolic pressure (less than 50 mmHg) or, in diabetics, absent peripheral pulses, in addition to rest pain or tissue necrosis (European Consensus on Critical Limb Ischaemia 1989, 1992). However, the validity of including what are fairly arbitrary ankle pressures in the definition of disease has been challenged (Thompson et al 1993, Tyrell and Wolfe 1993).

1.2 Atherosclerosis

Atherosclerosis is derived from the Greek words *athero* (meaning gruel or paste) and *sclerosis* (hardness). A normal arterial wall consists of two layers of smooth muscle, the tunicae intima and media, which are surrounded by a layer of adventitia. Athersclerosis

involves deposition of lipids, cellular waste products, calcium and fibrin in the tunica intima, resulting in the formation of atherosclerotic plaque.

The earliest precursors of atherosclerotic plaque are thought to be 'fatty streaks', deposits of lipid in the tunica intima which can be seen in the aorta and large arteries during childhood (McGill 1968). In some people the disease progresses rapidly in their third decade, while in others it does not become threatening until they are in their fifties or sixties.

Exactly how atherosclerosis begins or what causes it is not known, but some theories have been proposed. It is thought that atherosclerosis may begin because the inner lining of the artery (endothelium) becomes damaged, resulting in denuding of the intima. Three of the possible causes of damage to the arterial wall are;

- elevated levels of cholesterol and triglyceride in the blood
- high blood pressure
- cigarette smoke

This damage results in a proliferation of smooth muscle cells to form the simple or fibrous plaque, which, over time, progresses to a complicated plaque. Lipids, fibrin, platelets, cellular debris and calcium are deposited in the artery wall. These substances may stimulate the cells of the artery wall to produce still other substances that result in proliferation and migration of macrophages and vascular smooth muscle cells into the forming atherosclerotic plaque. At the same time, lipids build up within and around these

cells, which also form connective tissue (Ross 1986a, Ross 1993).

One recent theory on the early events in atherogenesis suggests that excess lipoproteins in the blood are trapped within the arterial wall where they are subsequently oxidized. These modified lipoproteins are taken up by macrophages or smooth muscle cells more rapidly than normal to form cholesterol-laden foam cells (Steinberg et al 1989, Leake 1993). This, in turn, leads to the deposition of connective tissue cells and other elements (Klatt and Esterbauer 1996). Platelets may also have a particularly important role in atherosclerosis; one of the prostaglandins which they form may damage arteries and platelet-derived growth factors can stimulate the growth of smooth muscle cells. Platelets are normally present in the arterial wall, but their abnormal growth and accumulation is believed to be one of the earliest events in the atherosclerosis process (Ross 1986b).

Whatever the underlying cause of the atherosclerotic process, the innermost layer of the artery becomes markedly thickened by the accumulating cells and surrounding material. If the wall is thickened sufficiently, the diameter of the artery will be reduced and the amount of blood decreased, thus decreasing the oxygen supply. In addition, two 'acute' events may occur;

- haemorrhage into the plaque: intraplaque haemorrhage is clinically important as it may erupt through the surface of the plaque, producing an embolisation of platelets, fibrin and cholesterol

- formation of a blood clot (thrombus) on the plaque surface following loss of surface integrity

If either of these occurs and blocks the entire artery, an acutely ischaemic leg may result (Badimon et al 1992).

1.3 Measurement of peripheral arterial disease

Symptomatic peripheral arterial disease is most commonly measured in epidemiological surveys using the World Health Organisation (WHO)/Rose intermittent claudication questionnaire (Appendix I). This questionnaire was devised in 1962 by Professor G. Rose to diagnose intermittent claudication in epidemiological surveys, and was subsequently adopted by the WHO for use worldwide (Rose 1962). The severity of intermittent claudication can be graded using the questionnaire – Grade 1: symptoms occur while walking 'at an ordinary pace on the level' and Grade 2: symptoms occur while walking 'uphill' or in a 'hurry'. It can also be used to identify 'possible claudicants', i.e. those with exercise leg pain not present at rest, but not otherwise fully concordant with the full WHO criteria (Criqui et al 1985). Unfortunately, although the WHO questionnaire is very specific, it is only moderately sensitive, and a more accurate alternative devised in Edinburgh is now becoming more widely used (Fowkes 1988a, Leng and Fowkes 1992).

Peripheral arterial disease can also be measured by clinical tests such as the ankle brachial pressure index (ABPI), the ratio of systolic blood pressure in the ankle to that in the arm. This can be easily, quickly and reproducibly measured using a portable Doppler probe and sphygmomanometer (Fowkes et al 1988). In small studies on hospital patients, an ABPI of less than 0.9 has been shown to be up to 95% sensitive in detecting angiogram positive disease (Yao et al 1969, Hummel et al 1978, Ouriel et al 1982) and a ratio of greater than 0.9 was almost 100% specific in identifying supposedly healthy subjects (Fowkes 1988a). However, these studies mostly used selected hospital patients who were symptomatic and it is not known how the ABPI would correlate with disease in asymptomatic subjects. In such subjects, the ABPI cannot be assumed to be a valid indicator of disease on its own.

In some subjects known to have significant peripheral arterial disease, blood pressures may be normal at rest but may become abnormal after exercise (exercise stress test) or an equivalent stress such as that caused by obstructing blood flow (reactive hyperaemia stress test). The exercise stress test is usually performed on a treadmill; following a standard amount of exercise, subjects with peripheral arterial disease show a significantly greater fall in ankle pressure and have a longer recovery time than normal subjects (Fowkes 1988a). However, practical problems due to the use of heavy equipment and inability of some patients with co-existing cardiac or mobility problems to complete the test restricts the usefulness of this test in epidemiological surveys.

In the reactive hyperaemia test, ankle systolic pressure is measured after the release of a pneumatic cuff occluding arterial blood flow just above the knee. The systolic pressure in patients with peripheral arterial disease is reduced two to three times further and takes longer to return to pre-occlusion levels than in healthy subjects (Johnson 1975, Hummel et al 1978). In general, using a cuff pressure about 50 mmHg above systolic for only 3 minutes to occlude blood flow causes minimum discomfort to the patient while still resulting in a significant post-occlusion alteration in blood flow. Measuring the blood pressure at between 15 and 30 seconds after the cuff has been released has been shown to be the optimum time to distinguish between subjects with angiogram positive disease and controls; in this situation the test is over 95% sensitive in detecting disease (Johnson 1975, Fowkes 1988a). The test may be particularly useful for detecting subjects with peripheral arterial disease in whom the presence of medial arterial calcification (as may occur in diabetes) can render measurement of the ABPI an unreliable marker of disease.

Techniques used to assess disease severity include angiography, duplex scanning and walking tests. Angiography, in which injection of contrast medium into a peripheral artery allows visualization of the lower limb arterial system, is considered the 'gold standard' against which to evaluate all other measures of peripheral arterial disease. However, angiography is expensive and time-consuming to perform. It is also not without risk to the patient, and for this reason it is not suitable for use in population surveys. Angiography and duplex scanning have the advantage of localizing abnormal arterial segments and categorizing disease severity at specific sites. With angiography as

the gold-standard, peak systolic velocity ratios greater than 2 on duplex are 95% specific and at least 85% sensitive at detecting stenoses affecting more than 50% of the arterial lumen diameter (Leng 1993).

Treadmill exercise testing, in which patients walk at a defined speed and slope until the onset of claudication pain (claudication distance) or a maximum level of claudication pain is reached (maximum walking distance) is well accepted by both patients and physicians and is widely used as a clinical test of disease severity. However, the reproducibility of treadmill testing is poor and results should be interpreted with caution (Ouriel et al 1982).

1.4 Incidence and prevalence

The incidence of intermittent claudication (the development of new cases in a population initially free of disease) has only recently begun to be reported in samples of the general population which were not highly selected. In middle-aged subjects, annual incidences of 1.0 to 10.1 per 1000 have been reported in four population studies (Bainton et al 1994, Bowlin et al 1994, Leng et al 1996, Murabito et al 1997). The incidence of disease was higher in men than in women (Murabito et al 1997) and generally increased with age (Bainton et al 1994, Bowlin et al 1994, Murabito et al 1997).

The prevalence of intermittent claudication (the proportion of individuals with disease at

any given time) is easier to measure and has been reported more widely. However, comparison between populations can be difficult due to highly variable criteria used by different studies to diagnose disease. Table 1.1 summarises prevalence figures from population studies, all of which used the WHO or Edinburgh claudication questionnaire as standard measures of disease, allowing reasonable inter-population comparisons. The prevalence of intermittent claudication varied widely, ranging from 1.0% to 7.7% in middle-aged men and from 0.4% to 4.6% in middle-aged women. Prevalence increased with age and, although inconsistently reported, appeared to be approximately 1.5 to 2 times more common in men than in women.

Accurate figures on the incidence of critical limb ischaemia are not available. The Second European Consensus Document on Chronic Critical Limb Ischaemia (1992) estimated an annual incidence of between 50 and 100 per 100,000 population, whereas recent data from Oxford suggested an incidence of 30 per 100,000 per year (Collins 1992). From a national audit, the Vascular Surgical Society estimated that 20,000 patients presented annually in the United Kingdom and Ireland, equating to 40 per 100,000 per year (Harris et al 1995). Critical limb ischaemia is more common in men than women (approximately 1.5:1), and the prevalence generally increases with age (Harris et al 1995).

The wide variety of methods used to detect asymptomatic disease in population studies makes it particularly difficult to compare figures for disease prevalence. However, in general, the prevalence of disease detected by noninvasive procedures is at least three

times greater than the prevalence of intermittent claudication, with a similar distribution by age and sex (Dormandy et al 1999a).

1.5 Prognosis

Peripheral arterial disease stabilizes soon after onset in approximately 75% of patients, and only 25% of claudicants will deteriorate to rest pain or gangrene (Dormandy et al 1999b). A subjective improvement in symptoms does not necessarily indicate regression of atherosclerosis, but may simply represent psychological and physiological adaptation to the ischaemia, such as the development of a collateral circulation. Only 3% to 22% of those claudicants presenting to a doctor ever require reconstructive arterial surgery, and as few as 1% may undergo amputation (Leng and Fowkes 1993). In addition to local symptoms, claudicants have an increased prevalence of coronary artery disease and cerebrovascular disease; coronary disease is the most common cause of death in subjects with peripheral arterial disease (Dormandy et al 1999b).

More than 90% of patients with critical limb ischaemia undergo major amputation, arterial reconstruction or angioplasty over the twelve months following presentation (Harris et al 1995). Amputation leads to loss of mobility and independence, psychological morbidity and reduced life expectancy. If untreated, critical limb ischaemia is often fatal. Even if treated, patients with critical limb ischaemia have a high risk of

dying, with a mortality in excess of 10% per year, usually from coexistent coronary artery disease and cerebrovascular disease (Wolfe 1986, DeWeese et al 1993, Tyrell and Wolfe 1993).

1.6 Risk factors

It is likely that many of the risk factors for peripheral arterial disease are common to all atherosclerotic conditions, including coronary artery disease and ischaemic stroke. However, there is evidence that peripheral arterial disease has its own risk factor profile in terms of the relative importance of such atherosclerotic risk factors.

A summary of selected risk factors implicated in the pathogenesis of peripheral arterial disease is given in Table 1.2. For many of these, however, much of the evidence comes from the study of coronary artery disease and their role in peripheral arterial disease is uncertain. The impact on peripheral arterial disease of the 'traditional' cardiovascular risk factors (smoking, hypertension and hyperlipidaemia) and/or those included as possible confounding factors in the projects presented in this thesis are discussed below. Diabetes, plasma insulin and steroid sex hormones are reviewed in detail in chapter 2.

1.6.1 Smoking

Cigarette smoking is the single most powerful risk factor for peripheral arterial disease (Schroll and Munck 1981, Fowkes 1988b, Fowkes et al 1992). Three quarters of cases of intermittent claudication may be attributable to smoking (Kannel and McGhee 1985), which is a more important risk factor for the development of peripheral arterial disease than it is for ischaemic heart disease. Thus, cigarette smoking can result in a seven-fold increase in the risk of peripheral arterial disease (Heliovaara et al 1978, Hughson et al 1978), compared with a two-fold increase in the risk of coronary artery disease (Doll et al 1976, Doll et al 1980).

The pathophysiological mechanisms by which smoking results in the development of atherosclerotic disease are unknown. Suggested mechanisms include endothelial disturbance, changes in fibrin formation and turnover, altered blood rheology, changes in lipids and lipoproteins and reduced availability of antioxidants. Thus, smokers have been shown in some studies to have increased plasma levels of von Willebrand factor (a marker of endothelial dysfunction), raised plasma fibrinogen (the precursor of fibrin) and elevated haematocrit (Ogston et al 1970, Meade et al 1987, Blann 1991, Smith et al 1993), together with altered blood lipid and lipoprotein profiles (Craig et al 1989) and reduced circulating antioxidants (Duthie et al 1990). However, in the Edinburgh Artery Study, the combined effect of smoking on these risk factors explained only part of its influence on peripheral arterial disease, and the majority of the effect appeared to be due to other

mechanisms (Price et al 1999). The latter could include a direct toxic effect of whole smoke, nicotine and/or carbon monoxide on endothelial cells (Krupski 1991), increased platelet reactivity and aggregability (Lassila et al 1988), and/or a detrimental effect of the elevated white blood cell count found consistently in smokers (Corre et al 1971, Friedeman et al 1973).

1.6.2 Hyperlipidaemia

Various lipids and lipid fractions have been implicated in the pathogenesis of peripheral arterial disease, including total serum cholesterol, lipoprotein subfractions and plasma triglycerides. It seems fairly certain that raised serum cholesterol increases the risk of disease. Thus in most (Hale et al 1988, Fowkes et al 1992), though not all (Gofin et al 1987), cross-sectional population studies, total serum cholesterol was found to be independently associated with peripheral arterial disease. In prospective studies, serum cholesterol was found to be an independent risk factor for intermittent claudication (Kannel and McGhee 1985) and to correlate with ankle brachial pressure indices (Schroll and Munck 1981).

In recent years, interest has concentrated on the lipoprotein subfractions of cholesterol. Low density lipoprotein cholesterol is the main carrier of cholesterol from the liver to hepatic and peripheral tissues and tends to reflect the role of total cholesterol as an atherosclerotic risk factor. High density lipoprotein cholesterol picks up cholesterol from peripheral tissues and returns it to the liver, thus acting in opposition to low density lipoprotein cholesterol (Gotto

1990). Reduced levels of high density lipoprotein cholesterol have been found in the vast majority of hospital cases of peripheral arterial disease compared with control subjects (Leng and Fowkes 1993), and are also associated with an increased severity of disease (Beach 1979, Jacobson et al 1984). In the few community studies in which high density lipoprotein cholesterol has been examined, there is some support for lower levels of the lipoprotein in those with disease (Pomrehn et al 1986), but other studies have shown no significant relationship (Gofin et al 1987). However, in the Edinburgh Artery Study there was a strong inverse relationship between high density lipoprotein cholesterol levels and intermittent claudication, which persisted on adjustment for other lipids, obesity, smoking, diabetes and alcohol consumption (Fowkes et al 1992).

Elevated levels of serum triglycerides have been identified in patients with peripheral arterial disease in numerous case control studies (Leng and Fowkes 1993). Similarly, the majority of cross-sectional (Hughson et al 1978, Schroll and Munck 1981, Pomrehn et al 1986, Hale et al 1988, Fowkes et al 1992) and prospective (Da Silva and Widmer 1980, Schroll and Munck 1981) population studies have shown a univariate association with triglyceride levels. However, whether serum triglycerides are important in the development of disease in their own right is uncertain, since the relationship generally disappears when multivariate analysis is undertaken to adjust for other risk factors, including smoking, blood pressure, and other lipids (Schroll and Munck 1981, Pomrehn et al 1986). Evidence from the Edinburgh Artery Study suggests that the strong univariate relationship between triglycerides and peripheral arterial disease can mostly be explained by the correlation between triglyceride levels and non-high

density lipoprotein cholesterol (Fowkes et al 1992), comparable with the situation for serum triglycerides and coronary artery disease (Austin 1989).

1.6.3 Hypertension

Elevated blood pressure has been associated with the development of peripheral arterial disease in many studies. Most case control studies and cross-sectional surveys show associations with only systolic pressure (De Backer et al 1979, Schroll and Munck 1981, Jacobson et al 1984, Gofin et al 1987, Hale et al 1988) although a few implicate both systolic and diastolic pressure (Reid et al 1966, Hughson et al 1978), have conflicting results (Bothig et al 1976) or show no association (Reunanen et al 1982). However, as elevated blood pressure may be secondary to atherosclerotic changes in blood vessels, its role in the aetiology of peripheral atherosclerosis can only be clearly determined in prospective studies. Unfortunately, the results from cohort studies are not consistent: one reported a three-fold increased risk of intermittent claudication at 26 years follow-up, primarily associated with a raised systolic pressure (Kannel and McGhee 1985); another found that both initial systolic and diastolic pressures were correlated with the ankle pressure index after 10 years (Schroll and Munck 1981), whereas a third did not find any association between blood pressure and the development of intermittent claudication (Da Silva et al 1979). Therefore, the importance of elevated blood pressure as a risk factor for peripheral arterial disease has not been clearly established in epidemiological studies.

1.6.4 Obesity

It is generally assumed that people who are obese are at an increased risk of arterial disease. This is primarily due to reported associations between body mass index (as a measure of overall obesity) and coronary artery disease, since investigation into the relationship between obesity and other forms of arterial disease is scarce. However, even in studies on coronary artery disease, an elevated body mass index has often not been significantly related to the risk of disease (Bradley 1982, Keys et al 1984) or only in very large samples (Manson et al 1990). Recently, some studies have suggested that the localisation rather than the degree of obesity is the important factor. Thus, in women, an increased ratio of waist circumference to hip circumference (waist hip ratio) was a significant risk factor for subsequent mortality from myocardial infarction independent of body mass index, whereas body mass index itself was not a significant independent risk factor (Lapidus et al 1984, Bengtsson et al 1993). Such an 'android' distribution of adipose tissue, with relative abdominal adiposity, has now been shown to be a risk factor for coronary artery disease in both men and women (Lapidus and Bengtsson 1988, Larsson 1988, Thompson et al 1991). Some of this effect may be due to the association between visceral fat accumulation and a variety of risk factors, including raised low density lipoprotein cholesterol and triglycerides, reduced high density lipoprotein cholesterol, hypertension, hyperinsulinaemia and insulin resistance (Després et al 1990). Although the waist hip ratio is often used to assess degrees of abdominal fat, the results of computed tomography indicate that it is more likely to be the amount of intra-abdominal or visceral fat that predicts coronary artery disease (Fujioka et

al 1987, Peiris et al 1988).

The role of obesity and fat distribution in peripheral arterial disease is uncertain. In the Edinburgh Artery Study, body mass index was not related to either symptomatic or asymptomatic peripheral arterial disease (Fowkes et al 1992). This was consistent with findings from a number of other case control and cross-sectional studies (Reunanen et al 1982, Pomrehn et al 1986, Vigna et al 1992, Newman et al 1993), although in Italy, the prevalence of peripheral arterial disease was associated with a raised body mass index in women but not in men (Novo et al 1992). In the Speedwell prospective study, body mass index did not predict the development of intermittent claudication in middle aged men from the general population (Bainton et al 1994). However, after 5 years of follow-up, a raised body mass index was (weakly) associated with an increased incidence of intermittent claudication in Israeli men (Bowlin et al 1994). Measures of adiposity other than body mass index have not been widely considered. In a single study on elderly Japanese American men, there appeared to be a 'U-shaped' relationship between waist hip ratio and a reduced ankle brachial pressure index (less than 0.9), with higher abnormality rates in the lowest and highest quintiles of waist hip ratio than in the midrange. However, this relationship was not statistically significant and was similar to that found for body mass index (Curb et al 1996).

1.7 Background to project

The Edinburgh Artery Study was established in 1988 to address the relative lack of epidemiological research into peripheral arterial disease. The initial cross-sectional survey involved clinical examination of 1592 men and women, aged 55 to 74 years, selected at random from the general population of Edinburgh. In the prospective phase of the study, subjects were followed up for cardiovascular events and death, and were invited to a 5-year follow-up clinic where they underwent further clinical examination.

Analysis of the data collected on disease parameters and potential risk factors has led to a greater understanding of the distribution and aetiology of peripheral arterial disease in the general population. However, one area which has not received a great deal of attention (either in the Edinburgh Artery Study or elsewhere), is the role of endocrine risk factors. For example, diabetes is a particularly well-recognised risk factor for peripheral arterial disease, but the reasons why are unknown. Whether or not hyperinsulinaemia (a characteristic of many subjects with diabetes) might affect the development of peripheral arterial disease is uncertain. Similarly, although peripheral arterial disease is more common in men than in women, the role of endogenous steroid sex hormones in the aetiology of peripheral arterial disease has not been investigated

In view of this lack of information, it was decided to investigate the relationship between several endocrine risk factors (diabetes, plasma insulin and steroid sex hormones) and

peripheral arterial disease in the Edinburgh Artery Study. Data was already available on the diabetic status of study participants and levels of 'traditional' cardiovascular risk factors. This allowed the author to undertake original analysis on the relationship between these factors and the development of peripheral arterial disease without further data collection. However, plasma insulin and steroid sex hormone levels had not previously been measured. It was not considered practicable, on the grounds of cost and 'over-investigation' of the study population, to carry out investigations on the whole study population. Instead, a more feasible option was to perform a case control study on selected subjects; this was undertaken by the author during 1995, using subjects who had attended the 5-year follow-up examination.

1.8 Aims and objectives

The overall aim of the work presented in this thesis was to investigate the association between peripheral arterial disease and a range of endocrine risk factors (diabetes, plasma insulin and steroid sex hormones).

The specific objectives were:

a) To determine whether the higher prevalence of peripheral arterial disease in subjects with diabetes or impaired glucose tolerance compared with non-diabetic subjects

could be explained by differing levels of 'traditional' risk factors, including;

i) Cigarette smoking

ii) Hypertension (systolic and/or diastolic)

iii) Dyslipidaemia (hypercholesterolaemia, hypertriglyceridaemia, raised low density lipoprotein cholesterol and/or reduced high density lipoprotein cholesterol)

iv) Elevated body mass index

b) To determine whether the following measures of circulating insulin and/or insulin resistance were associated with peripheral arterial disease in non-diabetic men and women and, if so, whether this was independent of other cardiovascular risk factors;

i) Fasting plasma insulin levels

ii) Plasma insulin levels one hour after a standard oral glucose load

iii) Insulin resistance

c) To determine whether the following endogenous steroid sex hormones were associated with peripheral arterial disease in men and/or women;

i) Total plasma testosterone

ii) Free plasma testosterone

iii) Plasma sex hormone-binding globulin

iv) Plasma oestradiol

v) Plasma oestrone

The rationale for measuring free testosterone in addition to total concentration was that, while there is an overall high correlation between the two measures under normal circumstances, it is possible that the percentage of hormone which is free in the circulation may differ between subjects with and without peripheral arterial disease (for example, due to variations in the affinity of testosterone for sex hormone-binding globulin). In addition, testosterone parameters have been found to change from around age 40, with a reduction in free testosterone levels and an increase in sex hormone-binding globulin levels of about 1.2% per year, so that total testosterone levels may not adequately reflect the true level of bioavailable testosterone in older men (Gray et al 1991).

It was not proposed to measure free oestrone because it binds only loosely to albumin and not at all to sex hormone-binding globulin. Neither was it proposed to measure free oestradiol. A smaller percentage of oestradiol is bound to sex hormone-binding globulin than is the case for testosterone and the relationship between concentration of the globulin and bioavailable oestradiol has not been as well established. The concentration of total oestradiol is low in postmenopausal women and men, such that any difference between subjects with and without peripheral arterial disease in the free fraction of hormone would be difficult to detect.

Table 1.1. Prevalence of intermittent claudication in population studies since 1970, using the WHO (or Edinburgh) Claudication Questionnaire

Reference (study)	Country	Study population (no.)	Age (yrs)	Prevalence (%) Men	Women
Gyntelberg 1973	Denmark	Selected company employees (5249)	40-59	2.0	-
			40-49	1.4	-
			50-59	3.1	-
Heliovaara et al 1978	Finland	Random population sample (1068)	50-74	7.7	-
De Backer et al 1979 (Belgian Heart Disease Prevention Project)	Belgium	Random population sample (8252)	40-59	1.4	-
			40-49	0.8	-
			50-59	2.3	-
Reunanen et al 1982	Finland	Random population sample (10962)	30-59	2.1	1.8
			30-39	0.6	1.2
			40-49	1.9	1.6
			50-59	4.6	2.9
Criqui et al 1985 (LRC Study)	USA	Selected population, including hyperlipidaemic group (613)	38-82	2.2	1.7
Pomrehn et al 1986	N. America	Random population sample (8326)	>20	1.0	0.4
Gofin et al 1987 (Jerusalem LRC Study)	Israel	Random population sample (1592)	>35	1.3	1.8
Laakso et al 1988	E. Finland	Random non-diabetic population samples (649 & 724)	45-64	5.6	1.8
	W. Finland			2.9	0.8
Fowkes et al 1991 (Edinburgh Artery Study)	Scotland	Random population sample (1592)	55-74	4.6	4.6
Smith et al 1991 (Scottish Heart Health Study)	Scotland	Random population sample (10042)	40-59	1.1	0.7
Dewhurst et al 1991	England	Random population sample (259)	65-95	7.0	4.0
Mittelmark et al 1993 (Cardiovascular Health Study)	USA	Random sample from 4 geographical communities (5201)	>65	2-4	1-3
Bainton et al 1994 (Speedwell Prospective Heart Disease Study)	England	Population sample of all men registered with 16 general practitioners (2348)	45-63	1.2	-
			50-54	1.0	-
			55-59	1.8	-
			60-63	2.2	-
Dong et al 1995 (Scottish Health Survey 1995†)	Scotland	Random population sample (7892)	16-64	1.7	1.9
			45-54	1.9	3.4
			55-64	5.0	3.0
Meijer et al 1998	Netherlands	Random population sample (6450)	>55	2.2	1.2

† Edinburgh Claudication Questionnaire (all other studies used WHO Questionnaire); LRC, Lipid Research Clinic

Table 1.2. Selection of suggested risk factors for peripheral arterial disease

Lifestyle factors
- Smoking
- Dietary deficiency
  - Antioxidant vitamins
  - Essential fatty acids
- Alcohol intake
- Lack of exercise

Diabetes mellitus and impaired glucose tolerance
- Type 1 and type 2 diabetes
- Impaired glucose tolerance/hyperglycaemia
- Hyperinsulinaemia/insulin resistance

Hypertension
- Raised systolic blood pressure
- Raised diastolic blood pressure

Obesity
- Body mass index
- Regional fat distribution/waist hip ratio

Haematologic factors
- Lipids and lipoproteins
  - Raised total serum cholesterol
  - Raised low density lipoprotein cholesterol
  - Reduced high density lipoprotein cholesterol
  - Hypertriglyceridaemia
  - Raised lipoprotein (a)
- Haemostatic factors
  - Raised plasma fibrinogen
  - Raised plasminogen activator inhibitor
  - Raised von Willebrand Factor
- Rheological factors
  - Raised blood viscosity
  - Raised plasma viscosity
  - Raised haematocrit
- Hyperhomocysteinemia
- Raised white cell count

Chapter 2

Literature review

Diabetes, insulin and steroid sex hormones

2.1 Diabetes mellitus

2.1.1 Classification and epidemiology

Diabetes mellitus is a clinical syndrome characterised by hyperglycaemia due to absolute or relative deficiency of insulin. Since the distribution of blood glucose in populations is unimodal, with no clear division between normal and abnormal, diagnostic criteria are necessarily arbitrary, and have been selected according to the degree of hyperglycaemia associated with microvascular complications (such as retinopathy, regarded as the hallmark of diabetes). Until very recently, the diagnostic criteria for diabetes mellitus involved a fasting plasma glucose of 7.8 mmol/l or greater and/or a level of 11.1 mmol/l or greater two hours after administration of a standard (75g) oral glucose tolerance test (National Diabetes Data Group 1979, WHO 1980). This World Health Organization criteria also recognised an intermediate zone of abnormal blood glucose levels called 'impaired glucose tolerance', diagnosed when the two hour plasma glucose value was

between 7.8 mmol/l and 11.1 mmol/l. In 1997, the American Diabetes Association proposed modifying the diagnostic criteria by lowering the fasting glucose at which diabetes can be diagnosed to 7.0 mmol/l (because a fasting value of 7.8 mmol/l defines a greater degree of hyperglycaemia than a 2-hour value of 11.1 mmol/l) (Report of Expert Committee 1997). This simple fasting sample is now preferred for diagnosis to the more complex 75g oral glucose tolerance test.

Diabetes has a worldwide distribution, although the prevalence varies considerably between different countries (King et al 1993, WHO 1994). Recent reports suggest that the prevalence of known diabetes in the United Kingdom is between 1% and 2% (Zimmet 1982, Gatling et al 1985, Neil et al 1987, WHO 1994). However, this is set to increase, since the incidence of both types of primary diabetes, type 1 diabetes (previously known as insulin-dependent diabetes mellitus or juvenile-onset diabetes) and type 2 diabetes (previously known as non-insulin-dependent diabetes mellitus or adult-onset diabetes) is rising worldwide (Kurtz et al 1988, National Center for Health Statistics 1994). In addition, almost 50% of cases of type 2 diabetes in the general population may remain undetected (Zimmet 1982). These factors mean that diabetes is a major and escalating public health problem.

In the United Kingdom, as in most European countries and North America, type 1 diabetes accounts for approximately 15% of all diagnosed cases of diabetes, while type 2 diabetes accounts for up to 85% (Gatling et al 1988). Other specific types of diabetes

result from certain genetic syndromes, surgery, drugs, malnutrition, infections, and other illnesses and account for only 1% to 2% of all diagnosed cases (National Diabetes Data Group 1979). Gestational diabetes, which develops in 2% to 5% of all pregnancies (Freinkel 1980), usually disappears when a pregnancy is over (Ratner 1993), although women who have had gestational diabetes are at increased risk for later developing type 2 diabetes (Damm et al 1992, Dornhorst and Beard 1993).

The causes of type 1 diabetes appear to differ from those for type 2 diabetes. The appearance of type 1 diabetes is suspected to follow exposure to an 'environmental trigger', such as an unidentified virus, stimulating autoimmune destruction of the ß cells of the pancreas in some genetically predisposed people (Szopa et al 1993, Atkinson and Maclaren 1994). For type 2 diabetes, risk factors include obesity, increasing age, family history of diabetes, physical inactivity and race (DeFronzo et al 1992). Twin studies also provide evidence for a genetic basis to the condition, but the exact mechanisms for development are unknown (DeFronzo 1997).

Whereas in type 1 diabetes the single fundamental defect is severe insulin deficiency (which, if untreated, results in hyperglycaemia), there appears to be two main pathophysiological defects in type 2 diabetes, namely altered insulin secretion and insulin resistance. Altered patterns of insulin secretion, including impaired meal-related insulin secretion, leads to exaggerated and prolonged postprandial hyperglycaemia, which is an important contributor to the overall loss of glycaemic control (Polonsky et al 1988).

Insulin resistance, defined as a diminished ability of insulin to exert its biological action across a broad range of concentrations, is high in those with type 2 diabetes (Ginsberg et al 1975, Reaven 1988, Yki-Järvinen 1995). However, resistance also varies widely in non-diabetic subjects, some of whom are as insulin resistant as diabetic subjects (Hollenbeck and Reaven 1987), suggesting that this defect alone cannot account for diabetes.

The relative importance of insulin resistance or ß-cell dysfunction in type 2 diabetes and whether one defect appears before the other in the natural history of the disease is uncertain and much disputed. Some studies indicate that insulin resistance appears before impaired insulin secretion (Bennett 1990); it is thought that the resultant hyperglycaemia stimulates insulin secretion, leading to hyperinsulinaemia so as to compensate and resist blood glucose increases. Because of ß-cell defects, maximal insulin secretory capacity is eventually reached; beyond that point (which corresponds to impaired glucose tolerance), insulin secretion declines and overt diabetes results (Figure 2.1) (Polonski et al 1996).

2.1.2 Diabetes mellitus and arterial disease

Cardiovascular disease is the most important cause of mortality and morbidity in patients with diabetes. Numerous prospective, population-based studies indicate that mortality from coronary artery disease is two to four times higher in subjects with type 2 diabetes than in comparable non-diabetic populations (Kannel and McGee 1979, Heyden et al

1980, Jarrett and Shipley 1985, Pyörälä et al 1987, Liao et al 1993, Sprafka et al 1993). The increased risk of disease associated with diabetes appears to be greater for women than for men (Kannel and McGee 1979, Heyden et al 1980, Pyörälä et al 1987, Liao et al 1993, Sprafka et al 1993). Cardiovascular mortality is less prominent in younger subjects with type 1 diabetes, but is still higher than in non-diabetic young adults (Barrett-Connor and Orchard 1985, Manske et al 1992). In addition, asymptomatic subjects with impaired glucose tolerance or borderline fasting hyperglycaemia are found to have an increased risk of coronary artery disease (Fuller et al 1979, Barrett-Connor et al 1984, Mykkänen et al 1992).

Peripheral arterial disease is also a well-recognized complication of diabetes mellitus. Tables 2.1 and 2.2 summarize population studies performed since 1970 which have measured the prevalence or incidence respectively of peripheral arterial disease in a predominantly type 2 diabetic population and a comparable non-diabetic population. The frequency of peripheral arterial disease was approximately 2.5 to 4 times higher in diabetic men and 3 to 6 times higher in diabetic women than in their non-diabetic counterparts, a difference which persisted after adjustment for variations in the age structure of the compared populations (Siitonen et al 1986, Walters et al 1992). A higher prevalence of peripheral arterial disease was found in previously undiagnosed diabetics (identified by screening the general population using a glucose tolerance test), in newly-presenting diabetics and in 'known' diabetics, already on treatment for the condition and with a range of diabetes duration. An increased risk of disease has also been reported in populations with type 1 diabetes (Walters et al 1992) and for more severe forms of

peripheral arterial disease, such as lower limb amputations (Jarrett 1991).

More recently, impaired glucose tolerance has been considered to be a real risk factor for peripheral arterial disease (Pyörälä et al 1987, Fujimoto et al 1991, Fowkes et al 1992, Uusitupa et al 1997). However, the evidence from various studies associating blood glucose levels with peripheral arterial disease is conflicting. In non-diabetic men referred to a vascular clinic, the degree of arterial disease at angiography was found to correlate with both fasting blood glucose and with blood glucose two hours after an oral glucose load (Kingsbury 1966). However, three cross-sectional studies found that the prevalence of intermittent claudication was not related either to a high fasting blood glucose, or to post-glucose levels (Hughson et al 1978, Reunanen et al 1982, Gofin et al 1987), and one found an inverse relationship (DaSilva et al 1979). Prospective studies have also produced conflicting results (DaSilva et al 1979, Schroll and Munck 1981, Kannel and McGhee 1985).

The cause of the increased risk of arterial disease in diabetics is unknown, although it is often assumed that adverse lipid and blood pressure profiles play an important role. Thus, type 2 diabetes is associated with hypertension (which may be twice as common in diabetes as in the non-diabetic population) and 'dyslipidaemia', consisting of low serum high density lipoprotein cholesterol and high triglyceride levels (Janka et al 1980, Kreines et al 1985, Breddin et al 1986, Kannel et al 1990, Uusitupa et al 1990, Mehler et al 1997, Al Zahrani et al 1997). Other blood lipids may also be adversely affected, including total and low density lipoprotein cholesterol (Breddin et al 1986, Kannel et al 1990, Uusitupa

et al 1990, Maser et al 1991, Walters et al 1992). In addition, some studies indicate that diabetic subjects tend to smoke more than non-diabetics (Paisey et al 1984, Breddin et al 1986, Uusitupa et al 1990). However, evidence to date suggests that such clustering of risk factors is only partly responsible for the increased coronary artery disease risk in diabetes (Fitzgerald and Jarrett 1991, Stamler et al 1993) and the extent to which they might explain the increased risk of peripheral arterial disease is unknown.

2.2 Insulin

2.2.1 Normal physiology and action

Insulin is a small protein composed of two amino acids chains connected to each other by disulphide bridges. It is synthesized by the ß cells within the Islets of Langerhans in the pancreas. Here, insulin preprohormone is cleaved to form proinsulin, which in turn is further cleaved to form insulin (Steiner et al 1972). Under normal circumstances, 95% of the hormone product is secreted into the circulation as insulin and less than 5% as unconverted proinsulin (Bell et al 1980).

The main stimulator of insulin release from the ß cells is glucose, such as that absorbed into the blood after a high carbohydrate meal (Ashcroft et al 1978). Following secretion, insulin circulates in the blood almost entirely in an unbound form; it has a plasma half-life averaging

only about 6 minutes so that it is mainly cleared from the circulation within 10 to 15 minutes. To initiate its effects in target cells, insulin first binds with a membrane receptor protein, a glycoprotein consisting of two extracellular α subunits and two β subunits, which are partly intracellular (Collier and Gordon 1991). This activates the receptor which initiates the insulin effects. Elevated insulin levels, as in type 2 diabetes, lead to 'down-regulation' of the receptor, where internalisation results in decreased numbers at the cell surface (Flier 1983). Except for that portion of the insulin that combines with receptors in the target cells, the remainder is mainly degraded in the liver and to a lesser extent the kidneys. This rapid removal from the plasma is important to ensure rapid turn off of the control functions of insulin.

The main function of insulin is the regulation of blood glucose levels. In normal subjects, blood glucose levels are maintained within relatively narrow limits at around 5 mmol/l by the balance between glucose entry into the bloodstream from the liver and from intestinal absorption after meals, and glucose uptake into the peripheral tissues. Thus low, basal insulin levels such as occurs between meals (Figure 2.2), suppress hepatic glucose output by inhibiting glycogen breakdown - glycogenolysis, and by inhibiting the formation of 'new' glucose – gluconeogenesis. Higher post-prandial concentrations of insulin stimulate rapid uptake, storage, and use of glucose by almost all tissues of the body, but especially the liver, muscles, and adipose tissue (Kruszynska 1997).

2.2.2 Role of insulin in arterial disease

Higher insulin levels in subjects with type 2 diabetes than in non-diabetics have been found for at least five years after diagnosis (Niskanen et al 1990), presumably due to the underlying insulin resistance and compensatory hyperinsulinaemia. In addition, individuals with type 1 diabetes are frequently hyperinsulinaemic due to peripheral administration of high-dose exogenous insulin (Elliott and Viberti 1993). The possibility that such raised plasma insulin levels might contribute to the increased risk of arterial disease in diabetes has stimulated interest in hyperinsulinaemia as a risk factor for arterial disease in general.

There is some evidence that raised plasma insulin levels may increase the risk of coronary artery disease. In two large prospective population-based studies, the Helsinki Policeman Study (Pyörälä et al 1979, Pyörälä et al 1985) and the Paris Prospective Study (Ducimetiere et al 1980, Fontbonne et al 1991), raised fasting and/or post-glucose insulin levels were associated with the subsequent development of coronary artery disease in middle-aged, non-diabetic men. However, recent analysis from a similar study in Busselton indicated that hyperinsulinaemia did not affect coronary mortality in a cohort of non-diabetic men and women (Welborn and Wearne 1979, Cullen et al 1983). A smaller prospective study in Nauru suggested an association between insulin and coronary artery disease, but this was based on the development of ischaemic electrocardiogram changes (Collins et al 1983). More recently, three major cross-sectional studies also found raised fasting or post-glucose insulin levels in men with coronary artery disease (Rönnemaa et al 1991, Modan

et al 1991, Mykkänen et al 1993).

The role of plasma insulin in the development of peripheral arterial disease has received less attention. Three early studies found some association between symptomatic peripheral arterial disease in non-diabetics and elevated post-glucose insulin levels, although not fasting levels (Welborn 1966, Sloan et al 1970, Sorge et al 1976). However, these were small hospital based studies with poor matching of cases and controls and no adjustment for potentially confounding factors. A more recent prospective study found that raised fasting insulin levels were associated with an increased risk of peripheral arterial disease in a population with type 2 diabetes but not in non-diabetics (Uusitupa et al 1990).

Raised insulin levels have been associated with a range of other cardiovascular risk factors, including hypertension (Manolio et al 1990, Modan et al 1991), raised total serum cholesterol, low density lipoprotein cholesterol and triglycerides and reduced high density lipoprotein cholesterol (Laakso et al 1989, Manolio et al 1990) as well as android obesity (Stern and Haffner 1986). Reaven termed such clustering of risk factors in subjects with an increased risk of atherosclerosis, 'Syndrome X', or insulin resistance syndrome, and further, suggested that insulin resistance was the underlying defect responsible for the other manifestations of the syndrome (Figure 2.3) (Reaven 1988). Other abnormalities which have been associated with syndrome X include raised concentrations of plasminogen activator inhibitor-1 and fibrinogen, which promote coagulation. The extent to which any association between hyperinsulinaemia and arterial disease may be dependent on these other risk factors is unclear.

2.3 Steroid sex hormones

2.3.1 Normal physiology

The steroid hormones, androgens (from the Greek *andros*, 'male') and oestrogens (from the Greek *oistros*, 'a gadfly'), regulate the differentiation and development of male and female reproductive organs, secondary sex characteristics, and behaviour patterns. Men and women each produce both androgens and oestrogens, but they differ markedly in the extent of production of each type of hormone (Siiteri and MacDonald 1973, Wilson 1975).

2.3.1.1 Androgens

Production

The term *androgen* means any steroid hormone that has masculinizing effects. The structure of testosterone, quantitatively the most important androgen, and the metabolic pathways by which testosterone is synthesized are summarized schematically in Figure 2.4 (Eik-Nes 1975). Pregnenolone is the major precursor of all steroid hormones and in each of the tissues that produce hormones, it is the rate-limiting conversion of cholesterol to pregnenolone that is regulated by various 'trophic' hormones produced by the pituitary (Eik-Nes 1975).

In men, the Leydig cells of the testes are the major source of androgens, including testosterone, together with lesser amounts of dehydroepiandrosterone and

androstenedione and very small amounts of dihydrotestosterone (Hammond et al 1977). At least five different androgens are also produced in the adrenals, though the total masculinizing activity of all of these is extremely slight (Nelson 1980).

In women, androgens are produced in the adrenals and ovaries; the major androgen is androstenedione, which can be converted to testosterone (or oestrogen) in the ovary and in extraglandular tissue (Nelson 1980, Lipsett 1986). About one fifteenth as much testosterone is secreted into the plasma of the female by the ovaries as into the plasma of the male by the testes (Lipsett 1986).

Protein binding and bioavailability

Following secretion, androgens are transported in blood bound to proteins. The two most important transport proteins are albumin, which binds androgens non-specifically, and a specific carrier protein known as sex hormone-binding globulin. Under ordinary circumstances, 1% to 3% of the testosterone in the circulation is free (unbound), up to 44% is bound to sex hormone-binding globulin (66% in women), and the remainder is bound to albumin (Dunn et al 1981). Binding of testosterone to albumin is loose and dissociation of this hormone fraction can occur within a capillary bed, such that nearly all albumin-bound testosterone is available for tissue uptake (Pardridge 1986). Thus the bioavailable circulating testosterone in normal subjects is equal to the free plus the albumin-bound hormone, which is determined primarily by the circulating concentration of sex hormone-binding globulin (Pardridge 1986). Testosterone circulates in the blood for about 15 to 30 minutes, by which

time it either becomes fixed to the tissues or degraded into inactive products that are subsequently excreted (Griffin and Wilson 1980). Much of the testosterone that becomes fixed to the tissues is converted within the target cells to the more biologically active hormone, dihydrotestosterone (Wilson 1975).

Testosterone levels in older men and women

In adult men, plasma testosterone levels are in the range 10 to 35 nmol/l. When older men are carefully screened to exclude major health problems or medication use, some studies demonstrate no fall in serum testosterone levels with age (Harman and Tsitouras 1980). Others demonstrate a clear decrease in mean serum testosterone levels (Tsitouras and Hagen 1984). However, most older men still have serum testosterone levels within the range considered to be normal for young men. Bioavailable testosterone is decreased in older men (Nankin and Calkins 1986, Tenover et al 1987). In one large study testosterone parameters began to change around age 40; free testosterone levels decreased by about 1.2% per year, and sex hormone-binding globulin levels increased by about 1.2% per year, so that total testosterone levels may not adequately reflect the true level of bioavailable testosterone in older men (Gray et al 1991).

In women, plasma testosterone levels are generally less than 3.5 nmol/l. This level falls postmenopausally, resulting in a wide range of values between approximately 0.05 and 1.5 nmol/l. This is because before the menopause, plasma androstenedione (the main precursor of testosterone) is derived equally from the adrenal and ovary; after the menopause the ovarian

contribution is minimal and plasma androstenedione levels decrease by 50% (Judd 1976).

2.3.1.2 Oestrogens

Oestrogens mainly promote proliferation and growth of specific cells in the body and are responsible for development of most secondary sexual characteristics of the female, uterine growth, thickening of the cervical mucosa, and development of the ductal system of the breast. Only three oestrogens are present in significant quantities in the plasma of the human female: β-oestradiol, oestrone and oestriol. The oestrogenic potency of oestradiol is approximately 10 times that of oestrone and 25 times that of oestriol. Considering these relative potencies, the total oestrogenic effect of oestradiol is usually many times that of the other two together. For this reason, oestradiol is considered to be the major oestrogen, though the oestrogenic effects of oestrone are far from negligible (Siiteri and Mac Donald 1973).

Production

The principal metabolic pathways by which oestrogens are synthesized, primarily in the ovaries, are illustrated in Figure 2.5. During synthesis, progesterone and the male sex hormone testosterone are generally synthesized first, mainly from cholesterol derived from the blood; then, before these can leave the ovaries, almost all the testosterone and much of the progesterone are converted into oestrogens (Gore-Langton and Armstrong 1994). In addition to formation of oestrogens in the ovaries, oestrogens are also formed by aromatization of

circulating androgenic precursors (in particular androstenedione secreted by the ovary and adrenal) in a variety of extraglandular tissues, including muscle, adipose tissue, hair follicles, liver and hypothalamus. Such peripheral aromatization is an important source of oestrogens, accounting for 40% of all oestrogen formation in menstruating women, 100% in postmenopausal women, and 85% in men (Baird et al 1968, Siiteri and MacDonald 1973).

The principal oestrogen secreted by the ovary is plasma oestradiol. Small amounts of oestrone are also secreted, but most of this is formed by extraglandular conversion of androgens, primarily in adipose tissue (Baird et al 1968). The rate of this aromatization is influenced by age, obesity, liver function, and thyroid function (Siiteri and MacDonald 1973). Oestriol is an oxidative product derived from both oestradiol and oestrone, the conversion occurring mainly in the liver (Fishman et al 1962).

Protein binding and bioavailability

Like testosterone, oestradiol is transported in the blood bound to plasma proteins. However, relatively less oestradiol is bound to sex hormone-binding globulin than is the case for testosterone (about 20% in normal men and 37% in normal women), most of the remainder binding loosely with plasma albumin (Dunn et al 1981, Rosner 1996). Although only 2% to 3% of oestradiol is free in the circulation, it is thought that part or all of the large albumin-bound fraction may be available for uptake by some tissues and therefore have biological activity (Pardridge 1981). Oestrone binds only loosely to albumin and hardly at all to sex hormone-binding globulin (Dunn et al 1981). The liver conjugates oestrogens and about one

fifth of these conjugation products are excreted in the bile while most of the remainder are excreted in the urine. Also, the liver converts the potent oestrogens, oestradiol and oestrone, into the almost totally impotent oestrogen oestriol.

Oestrogen levels in men and postmenopausal women

In men, circulating androgens can be aromatized to oestrone and oestradiol in extraglandular tissue (Siiteri and MacDonald 1973). Aromatization takes place in many tissues, the most significant of which is probably adipose tissue, and the overall rate of extraglandular aromitization increases with age and body size (Siiteri and MacDonald 1973). Small amounts of oestradiol are also secreted directly by the testes (MacDonald et al 1979).

In women circulating oestrogens are derived from two sources premenopausally (more than 60% secreted directly by the ovaries as oestradiol, and the remainder derived from extraglandular conversion of androgens to oestrogen). After the menopause, the ovarian contribution is reduced, and extraglandular formation of oestrone from adrenal androstenedione predominates (Siiteri and MacDonald 1973, Carr and MacDonald 1983). Thus, removal of the ovaries in postmenopausal women does not result in a further decline of oestrogen or androstenedione (Judd 1976). Because adipose tissue is a major site of extraglandular oestrogen production, oestrogen production is greater in obese than in thin postmenopausal women, and total oestrogen production in the massively obese may be as great or greater than in premenopausal women (Hemsell et al 1974, Edman and MacDonald 1978). Overall, there is considerable variation in estrone values postmenopausally (from

around 35 to 350 pmol/l); oestradiol levels tend to be much lower, with a substantial proportion of elderly women having a level below the usual limit of detection (Yen 1977).

2.3.1.3 Sex hormone-binding globulin

Sex hormone-binding globulin, also known as testosterone-estradiol binding globulin and sex steroid binding protein, is a circulating glycoprotein with a molecular weight of around 86,000. It is thought to be synthesized in the liver. In the circulation its biological function is the transport of steroid sex hormones. Each molecule has a single steroid-binding site (Rosner and Smith 1975, Cheng et al 1983) with a high binding affinity for testosterone and oestradiol. The circulating concentration of sex hormone-binding globulin is the major determinant of the relative amounts of testosterone (and probably oestradiol) that are non-protein-bound or bound to albumin. Thus, the ratio between serum total testosterone and sex hormone-binding globulin (commonly expressed as the 'Free Androgen Index') is directly equivalent to the circulating concentration of free testosterone (Nanjee and Wheeler 1985).

The production of sex hormone-binding gobulin appears to be increased by oestrogens and thyroid hormones, but decreased by androgens. Thus, circulating levels are increased during pregnancy, after the administration of synthetic oestrogens, in hyperthyroid patients, or after treatment with thyroid hormones (Rosner 1990). Conversely, slightly lower plasma concentrations in men than in women are considered to be a result of an androgen-induced

decrease in synthesis which occurs during puberty in boys (Blank et al 1978). In addition, insulin has been found to decrease sex hormone-binding globulin levels in men (Strain et al 1994, Pasquali et al 1995) and low levels of the globulin predict development of type 2 diabetes mellitus in men (Haffner et al 1996).

2.3.2 Role of steroid sex hormones in arterial disease

Interest in steroid sex hormones as possible risk factors for atherosclerotic disease arose from a number of observations, including the higher incidence of atherosclerotic conditions in men than in women and changes in coronary risk associated with the use of exogenous oestrogens. Work in this area, reviewed in the following section, has almost exclusively focused on coronary artery disease.

2.3.2.1 Oestrogens

Women

There is considerable 'indirect' evidence for an association between blood levels of oestrogens in women and risk of coronary artery disease. Surgical menopause in younger women increased coronary risk (Kalin and Zumoff 1990) and both naturally and surgically postmenopausal women had a greater risk of aortic atherosclerosis than age-matched premenopausal women (Witteman et al 1989). Cohort studies failed to show a consistently lower rate of coronary artery disease in premenopausal women compared to postmenopausal

women of a similar age, but this may have been influenced by difficulties with precise age-matching (Gordon et al 1978, Colditz et al 1987).

Coronary risk is also affected by the use of exogenous oestrogens as components of the oral contraceptive pill or hormone replacement therapy. In general, studies conducted when doses of oestrogen and progestogens in oral contraceptives were higher than they are now, indicated that users had an increased risk of vascular disease and myocardial infarction compared to non-users (Eaker et al 1993). More recent studies have suggested that low dose oral contraceptives reduce the risk of cardiovascular disease attributable to oral contraceptives and the newest preparations may even be protective (Eaker et al 1993). Interestingly, the increased risk of cardiovascular disease associated with older contraceptives ended with their discontinuation (Stampfer et al 1990) and it is possible that the immediate cause of coronary morbidity was more related to thrombosis than to atherogenesis.

The major epidemiological studies investigating oestrogen replacement therapy have been based upon unopposed conjugated equine oestrogen therapy, a 'natural' type of oestrogen which is considerably less potent than the lowest dose of synthetic oestrogen used in the oral contraceptive pill. In the vast majority of prospective studies, exogenous oestrogen use was found to reduce the incidence of coronary heart disease in postmenopausal women; a meta-analysis of the better, adequately controlled studies demonstrated a 50% risk reduction (Stampfer and Colditz 1990). Although these studies were not randomised trials and could potentially have been affected by selection bias, in many studies the risk factor profiles of users

and non-users were similar and in others they varied fairly equally between increased and decreased risk (Stampfer and Colditz 1990).

In addition to a potential, directly anti-atherogenic effect on blood vessel walls (Stevenson et al 1994), oestrogens may influence circulating levels of a number of other cardiovascular risk factors. Thus, in the majority of studies, post-menopausal women had higher total serum cholesterol, low density lipoprotein cholesterol, fibrinogen, and plasminogen activator inhibitor levels, and lower high density lipoprotein cholesterol levels than age-matched, premenopausal women (Matthews et al 1989, Scarabin et al 1993, Stevenson et al 1993). Exogenous oestrogen administration reversed these changes (Matthews et al 1989, Folsom et al 1991, Manolio et al 1993, Nabulsi et al 1993) and blood pressure (Lobo 1990) and fasting plasma insulin levels (Manolio et al 1993, Nabulsi et al 1993) were also reduced. Thus the advantageous effect of exogenous oestrogen on risk factor profiles provides a plausible mechanism for their cardioprotective effect in postmenopausal women.

Despite the fairly strong epidemiological evidence linking exogenous oestrogens to reduced coronary risk in postmenopausal women, the role of *endogenous* oestrogens in atherogenesis remains undetermined. In particular, both the formulation and concentration of the oral contraceptive pill and hormone replacement therapy are often incomparable to those of endogenous hormones. However, the cardioprotective effect of exogenous oestrogens in postmenopausal women (suggesting that the rate of development or progression of atherosclerosis may accelerate following oestrogen loss at the time of menopause) means that

we might expect endogenous oestrogen levels to be reduced in postmenopausal women with atherosclerotic disease. Only a single study has investigated the relationship between endogenous oestrogen levels and coronary artery disease in women; mean plasma oestrone levels did not differ significantly between postmenopausal women with and without coronary artery disease at angiography (Cauley et al 1994). However, it was not possible to measure oestradiol in this study and both the cases and control subjects presented with angina.

Men

Evidence surrounding a role for steroid sex hormones in male cardiovascular disease is confused. The administration of low dose exogenous oestrogens to male myocardial infarction survivors or those with carcinoma of the prostate had no effect on subsequent measures of cardiovascular disease (Stamler et al 1960, Oliver and Boyd 1961, Hanash et al 1970, The Coronary Drug Project Research Group 1973) or actually increased cardiovascular mortality (The Veterans Administration Cooperative Urological Research Group 1967, Blackard et al 1970, Henriksson et al 1987). This was despite an apparent increase in serum high density lipoprotein cholesterol and reduction in low density lipoprotein cholesterol levels (Russ et al 1955, Oliver and Boyd 1956). Raised endogenous oestradiol concentrations found following myocardial infarction, generally returned to normal after a year (Kalin and Zumoff 1990) and prospective studies failed to demonstrate a relationship between oestradiol levels and the subsequent development of coronary artery disease (Cauley et al 1987, Barrett-Connor and Khaw 1988, Phillips et al 1988, Eldrup et al 1989) or the extent of atherosclerosis at angiography (Okun et al 1985, Small et al 1985).

2.3.2.2 Androgens

Often linked to the supposition that oestrogens are cardioprotective is the hypothesis that testicular androgens are pro-atherogenic. Support for this hypothesis came from the observation of increased coronary risk in women with chronic anovulation, a condition associated with hypertestosteronaemia (LaVecchia et al 1987). However, in men, higher endogenous plasma testosterone concentrations were associated with an apparent improvement in the male risk factor profile.

Women

Whether or not the wide range of values for testosterone found in postmenopausal women affects their risk of atherosclerotic disease is unknown. In a limited number of studies, low high density lipoprotein cholesterol was associated with free testosterone levels (Haffner et al 1989) and the hyperandrogenicity of polycystic ovary disease (Wild et al 1985, Wild and Bartholomew 1988). Plasma insulin and blood glucose were positively related to free testosterone concentrations (Haffner et al 1988, Preziosi et al 1993) but no direct relationship was found between androgens and a variety of haemostatic factors, including plasma fibrinogen (Blomback et al 1992). Despite possible associations between testosterone and other cardiovascular risk factors, a single case control study found no difference in testosterone levels between women with angiographically-proven coronary artery disease and controls (Hauner et al 1994)

Men

In men, endogenous testosterone levels have been frequently associated with raised high density lipoprotein cholesterol levels (Heller et al 1981, Khaw and Barrett-Connor 1991, Phillips et al 1994) and reduced plasminogen activator inhibitor, plasma fibrinogen and insulin levels (Bonithon Kopp et al 1988, Yang et al 1993, Glueck et al 1993, Phillips et al 1994), indicating an *improvement* in risk factor profiles. However, those cross-sectional studies which found any variation in testosterone levels with coronary artery disease, demonstrated a *reduced* level of testosterone in subjects following a myocardial infarction or with angiographically-proven coronary artery disease (Barth et al 1983, Mendoza et al 1983, Phillips et al 1994) and prospective studies have failed to show any relationship between serum testosterone and the subsequent development of coronary artery disease (Cauley et al 1987, Barrett-Connor and Khaw 1988, Phillips et al 1988).

It is possible that an association between cardiovascular disease and steroid sex hormones in the limited number of studies available has been obscured by failing to take into account the entire hormonal 'milieu' and by not measuring free hormone levels (potentially especially important for testosterone). Moreover, the role of steroid sex hormones as risk factors for forms of cardiovascular disease other than coronary artery disease has not been investigated.

Figure 2.1 The 'Starling' curve of the pancreas

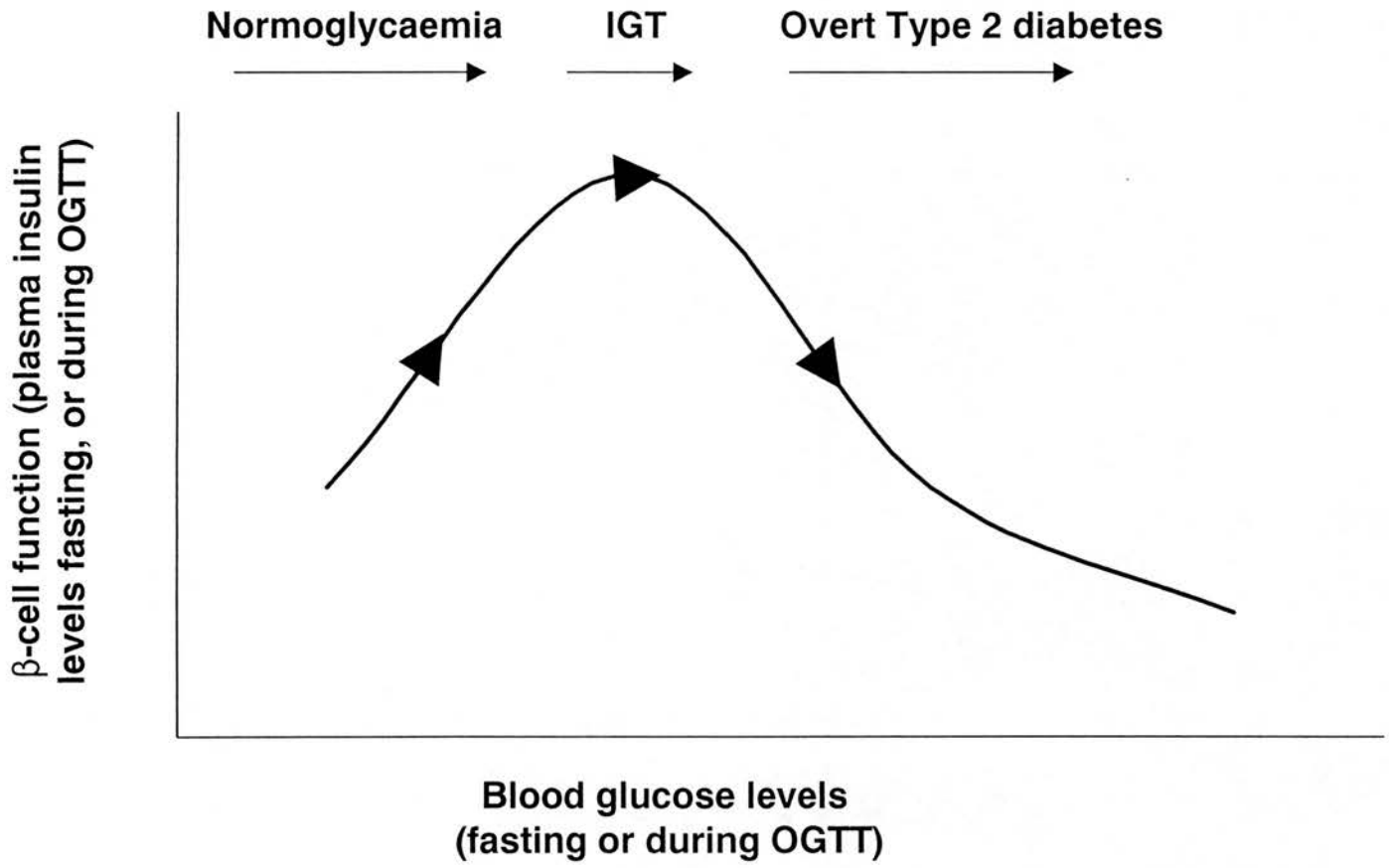


Table 2.1. Prevalence of peripheral arterial disease (intermittent claudication and/or asymptomatic disease) in population studies since 1970 comparing predominantly type 2 diabetic subjects with non-diabetics

Reference (study/country)	No. diabetic subjects	No. non-diabetic subjects	Age of subjects (years)	Diagnosis of diabetes	Diagnosis of peripheral arterial disease	Prevalence (%) Diabetic	Non-diabetic
Reunanen et al 1982 (Finland)	10962 in total study population		30-59	Clinical diagnosis	WHO IC Questionnaire	Men 5.0* Women 3.9*	Men 2.1* Women 1.8*
Siitonen et al 1986 (Finland)	133	144	45-64	Newly presenting type 2 diabetes (clinical diagnosis)	(i) WHO IC Questionnaire (ii) ABPI<0.9	Men 8.8† Women 5.9† Men 7.3† Women 1.2†	Men 6.7† Women 1.9† Men 2.3† Women 1.0†
Feskens et al 1992 (Zutphen Study, Netherlands)	46	230	>65	'Known' type 2 diabetes (clinical diagnosis)	WHO IC Questionnaire, gangrene, or chronic lower limb ulcer	10.9	5.2
Walters et al 1992 (England)	864	480	mean 67.7	'Known' type 2 diabetes (clinical diagnosis)	ABPI≤0.9	Men 22.2 Women 24.6	Men 11.0‡ Women 7.9‡
Mackaay et al 1995 (Hoorn Study, Holland)	173	288	50-75	'Known' diabetes (on treatment) or newly diagnosed on OGTT, predominantly type 2	Abnormal Doppler tracing and ABPI <0.9	31.8	18.4

* Approximate prevalence only (estimated from figure). Prevalence in 'non-diabetics' refers to prevalence in total population for this study (i.e. non-diabetics plus diabetics). Prevalence of PAD in diabetics 3.4 times higher in men and 5.7 times higher in women compared with non-diabetics.

† Prevalences age-adjusted;

‡ Odds ratio of PAD in type 2 diabetes vs non-diabetics after age adjustment 2.5 (men) and 3.2 (women).

PAD, peripheral arterial disease; IC, intermittent claudication; ABPI, ankle brachial pressure index; OGTT, oral glucose tolerance test.

Table 2.2. Incidence of peripheral arterial disease (intermittent claudication and/or asymptomatic disease) in population studies since 1970 comparing predominantly type 2 diabetic subjects with non-diabetics

Study/country (reference)	Population source and size	Age of subjects (years)	Duration of follow-up (years)	Diagnosis of diabetes	Diagnosis of peripheral arterial disease	5-year incidence	
						Diabetic	Non-diabetic
Herman et al 1977 (Israeli IHD Study)	Random sample of 10,059 male civil servants, 498 with diabetes	>40	5	'Known' cases of diabetes plus newly-diagnosed diabetes on OGTT at baseline	Clinical history of IC	9.3%	4.1%
Kannel and McGee 1979 (Framingham)	Cohort of 5209 men and women, 344 with diabetes	45-74	20	Clinical diagnosis of diabetes during follow-up	IC Questionnaire	Men 12.6/1000/year Women 8.4/1000/year	Men 3.3/1000/year Women 1.3/1000/year
Uusitupa et al 1990 (Finland)	Consecutive population sample of 133 diabetics and 144 randomly selected non-diabetics	45-64	5	Newly presenting Type 2 diabetes (clinical diagnosis) at baseline	WHO IC Questionnaire	Men 20.3% Women 21.8%	Men 8% Women 4.2%

IHD, ischaemic heart disease; IC, intermittent claudication; OGTT, oral glucose tolerance test.

Figure 2.2 Blood profiles of glucose and insulin in non-diabetic subjects

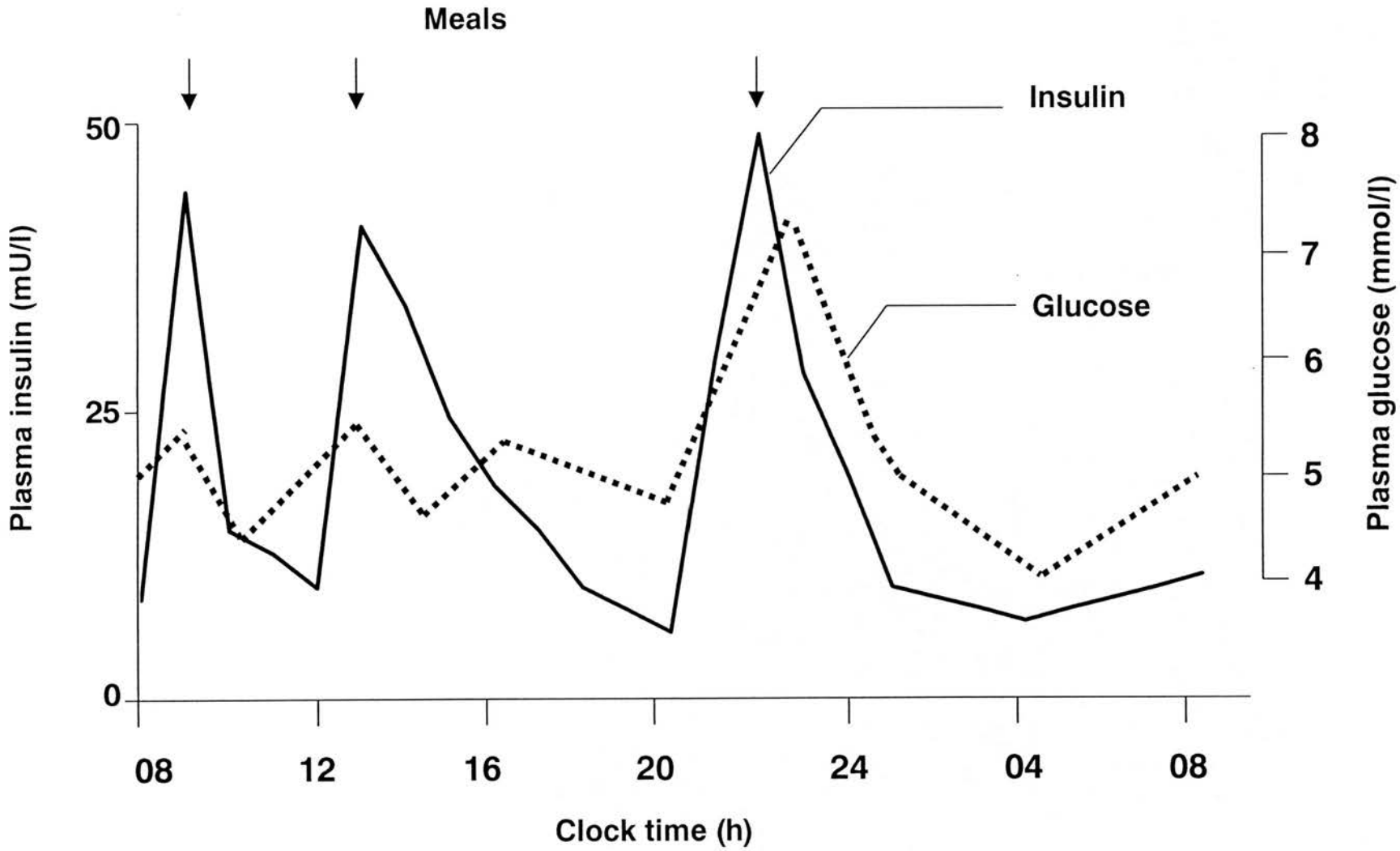


Figure 2.3 The 'insulin resistance syndrome' ('syndrome X')

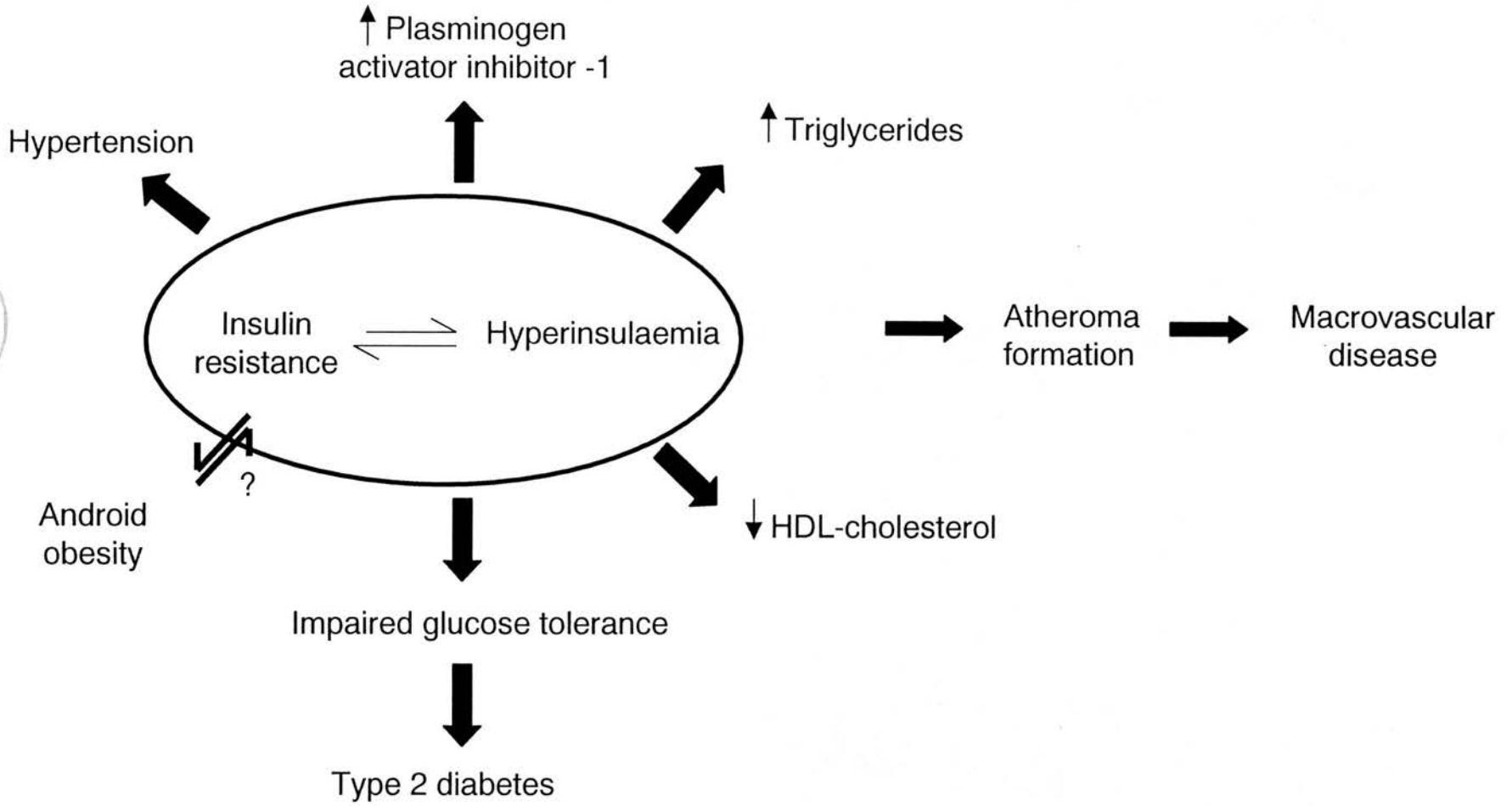


Figure 2.4 Pathways for androgen biosynthesis

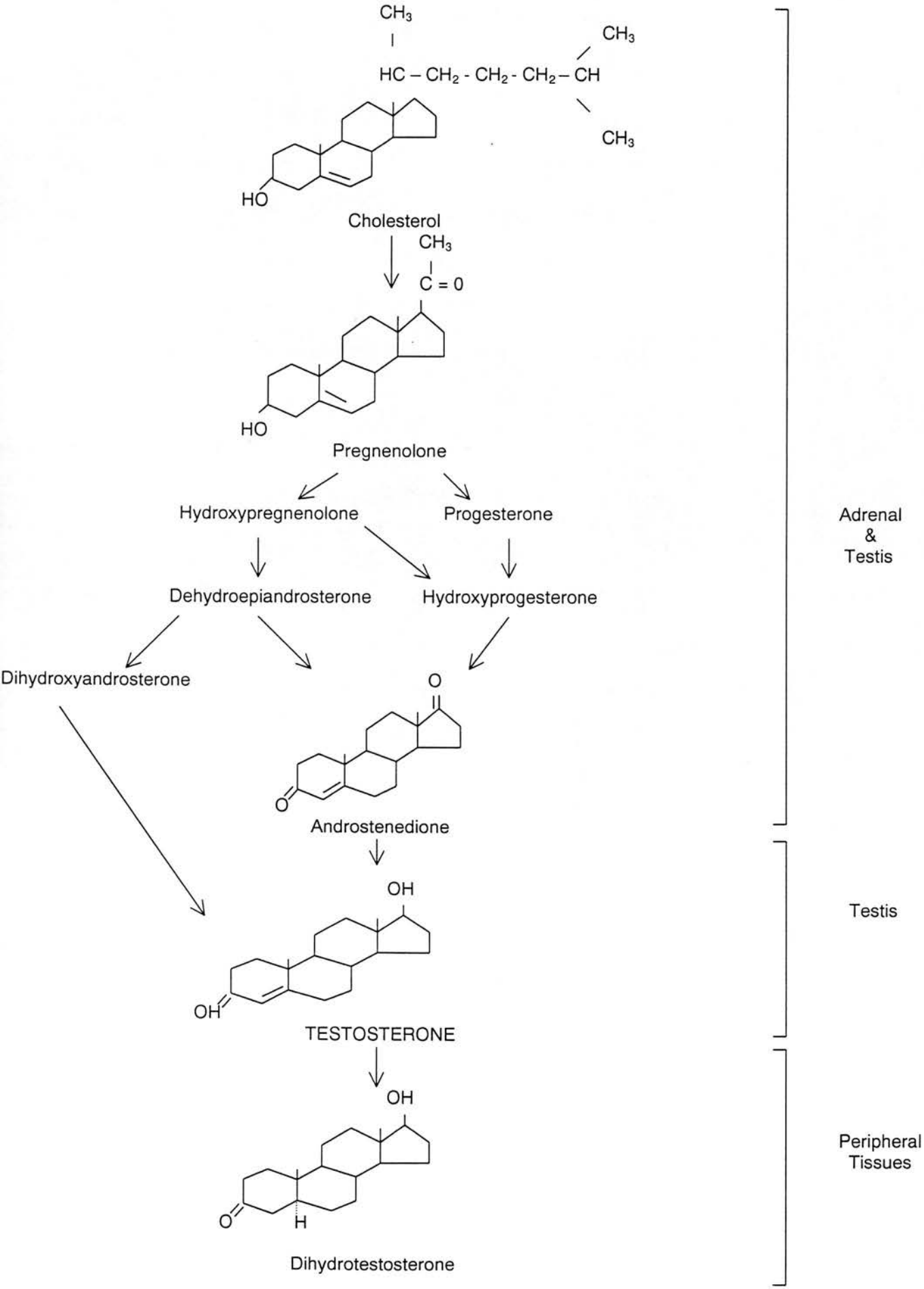
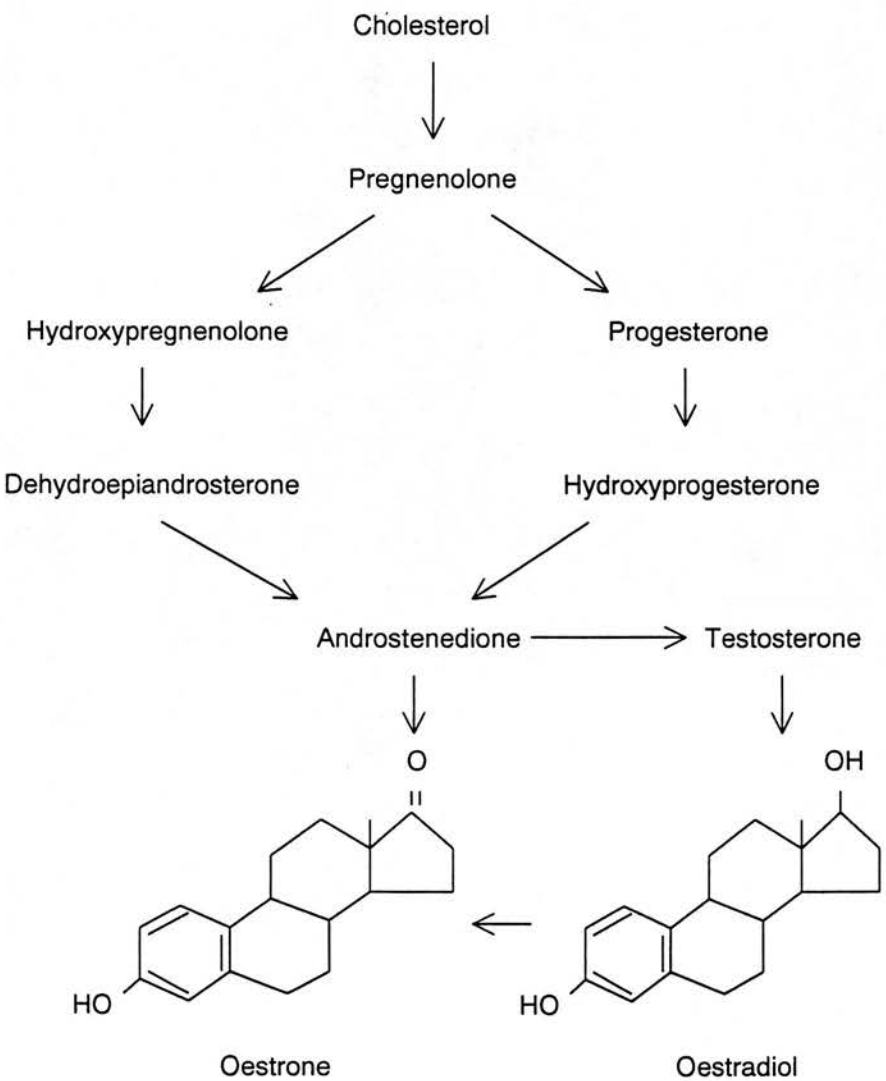


Figure 2.5 Pathways for oestrogen biosynthesis in human ovary



Chapter 3

Methods I

Edinburgh Artery Study: Relationship between diabetes and cardiovascular risk factors in the development of peripheral arterial disease

This chapter gives the methods of the Edinburgh Artery Study which are relevant to the work presented in this thesis. It also describes how data from the baseline survey was analysed to investigate the relationship between diabetes, cardiovascular risk factors and peripheral arterial disease. The methods of the case control study, based on subjects attending the 5-year follow-up phase of the Edinburgh Artery Study, and used to investigate the role of plasma insulin and steroid sex hormones in peripheral arterial disease, are given in chapter 4.

3.1 Study design and population

The Edinburgh Artery Study was established in 1988 as a cross-sectional survey designed to measure the prevalence of peripheral arterial disease and its risk factors in a representative sample of men and women from the general population. The target

population was Edinburgh residents aged 55-74 years. Participants were selected at random (in sex-specific, 5-year age bands) from the age sex registers of 11 general practices serving a range of socio-economic and geographic areas throughout the city. The aim was to include equal numbers of subjects from each 5-year age band and equal numbers of men and women. The sample size of 1500 participants was estimated on the basis of the number required to conduct a subsequent follow-up study with adequate power to detect differences in the incidence of cardiovascular events according to baseline characteristics. Subjects judged by the general practitioner to be unfit to participate (for example due to mental illness or terminal illness) were replaced by other randomly selected subjects (n=353; 13%).

Subjects were invited to attend a university clinic to complete a questionnaire and have a comprehensive medical examination. In an effort to maximise participation in the study, invitation letters were signed by the subject's general practitioner as well as the study director and were sent out after publicity in the local media. In addition, provision was made for home visits for subjects who would find it difficult to attend the clinics, and travelling expenses to and from the clinic were offered. Those whose invitation letters were returned by the post office were replaced by other randomly selected subjects (n=162; 6%). Once an affirmative reply was received, each subject was sent an appointment date, a map, and details about the examination. Those who did not respond at all were sent a second invitation letter. Affirmative responders who did not attend their appointment were offered a second appointment date, usually by telephone. Ethical approval was granted

for the study by Lothian Health Board Ethics Committee and informed consent was obtained from each subject.

To enable comparison of study participants and non-participants, some 20% of subjects in each practice who did not respond or attend were randomly selected for follow-up. Each was sent a letter enclosing a short questionnaire. Subjects not returning the questionnaire were telephoned or visited at home on up to three occasions at different times of the day and evening.

3.2 Clinical examination

Clinical examinations were held on weekday mornings from August 1987 to September 1988 at a specially set-up research clinic. Occasional out-of-hour sessions were arranged as required. Subjects were asked to fast overnight prior to attending for examination (from 11pm the previous evening), but were told that this did not apply if they were diabetic. They were also asked to refrain from smoking for two hours prior to the examination. Each subject underwent a clinical examination performed by one of two teams (each comprising a nurse and a technician). A self-administered questionnaire (Appendix I) was completed which included the World Health Organisation (WHO) intermittent claudication and angina questionnaires (Rose 1962). It also contained standard questions on social class, cardiovascular history (including recall

of a doctor's diagnosis of angina, myocardial infarction or intermittent claudication) and a detailed section on smoking habit. Social class coding (OPCS 1980) included the use of spouse's occupation for female married subjects. Retired subjects and those currently unemployed were coded according to longest occupation.

Following ten minutes' rest in the supine position, systolic and diastolic (Phase V) blood pressures were taken in the right arm using a Hawksley random zero sphygmomanometer. Ankle systolic blood pressures were taken in both legs using the random zero sphygmomanometer and a Sonicaid Doppler probe. Blood flow was detected where possible in the posterior tibial artery. The ankle brachial pressure index (ABPI) was calculated as ankle divided by brachial systolic pressure and the lesser ABPI in the two legs was used since disease often occurs unilaterally. In the reactive hyperaemia test which followed, ankle systolic pressure was measured 15 seconds after the release of a cuff occluding arterial flow just above the knee for four minutes at about 50 mmHg above systolic pressure. The timing was standardised using an electronic timer.

During the clinical examination, 20 mL of fasting blood was taken for measurement of (among other factors) serum total cholesterol, high density lipoprotein cholesterol and triglycerides, serum thiocyanate and plasma glucose. Following venepuncture, subjects consumed 75g glucose in the form of 335mL Solripe Gluctoza Health Drink (Strathmore Mineral Water Co). A second blood specimen was taken 2 hours after the oral glucose load. Subjects who, because of diabetes, attended for examination unfasted, did not

undergo the glucose tolerance test. Assays for serum lipids, thiocyanate and plasma glucose were performed in the laboratory on a Cobas Bio analyzer, Roche Products, using standard kits. The quality of the laboratory measures were checked by means of blind duplicate samples taken intermittently throughout the course of data collection.

A 12-lead electrocardiogram (ECG) was taken using a Hewlett Packard 'Pagewriter' electrocardiograph and coded independently by two specially trained researchers using the Minnesota code (Prineas et al 1982). In the event of disparity, the ECG was coded again by a third person. If the third code did not agree with either of the first two, the ECG was read by a consultant cardiologist and a final code agreed following discussion between the coders.

Standing height (without shoes) was measured to the nearest 5 mm using a free standing metal ruler on a heavy base. Weight without shoes and outer clothing was measured to the nearest 100g on digital scales (Soehnle).

Before conducting the main study, a pilot study of all clinical and laboratory procedures was carried out on 50 volunteers from the general public. The quality of the clinical measurements was checked before and during the study by repeat measurements taken intermittently by the study co-ordinator. Individual observer measurements were assessed for drift and variability studies of measurements carried out previously indicated that the level of variability was adequate for epidemiological purposes (Fowkes et al 1988, Fowkes et al 1992).

3.3 Data analysis

3.3.1 Classification of peripheral arterial disease

To enable comparison of subjects with and without peripheral arterial disease, the following criteria were used to separate subjects into 2 groups;

(i) Disease group - These were subjects with either major symptomatic peripheral arterial disease, defined as a positive WHO intermittent claudication questionnaire (grades 1, 2 and 'probable' - calf pain but one WHO criteria not fulfilled) or major asymptomatic disease. Major asymptomatic disease was defined as one of the following;

- an ankle brachial pressure index of 0.9 or less and a drop in ankle systolic blood pressure during the reactive hyperaemia test of more than 20%
- an ankle brachial pressure index of 0.7 or less
- a hyperaemic drop of more than 35%

(ii) Normal group – These were subjects with none of the above criteria plus an ankle brachial pressure index greater than 0.9 and a hyperaemic pressure reduction of less than 20%.

For each subject the lower of the two estimates of the ABPI in the left or right leg was used because disease often occurs unilaterally, and similarly, the greater reactive hyperaemia pressure drop of either leg was taken.

3.3.2 Classification of diabetes/impaired glucose tolerance

Subjects were separated into those with diabetes, impaired glucose tolerance or normal glucose tolerance using the responses to questions on diabetes in the self-administered medical questionnaire (questions 9 and 10, Appendix I), and the results of the oral glucose tolerance test. In question 9 of the questionnaire, subjects were asked whether they had ever been told by a doctor that they had diabetes (sugar disease). In question 10, they were asked whether they were on any regular medical treatment from a doctor, including (amongst other common medications) insulin injections and tablets for diabetes. Space was also provided for subjects to list any other treatments not previously specified. A fasting plasma glucose and a plasma glucose measurement 2 hours after a standard oral glucose tolerance test were also available for the majority of subjects, but not on those who, because of diabetes, did not fast overnight or undergo the test.

Subjects were classified as suffering from diabetes mellitus if:

(i) they had been told by a doctor that they suffered from diabetes (answered 'yes' in question 9) *and* were receiving either insulin or oral therapy (answered 'yes' in question 10 or wrote the name of medication used in diabetes), or

(ii) because of a doctor diagnosis of diabetes (answered 'yes' in question 9), they did not undergo the oral glucose tolerance test ('missing' entered on database) - these subjects were classified as diabetic irrespective of whether or not they were on insulin or oral therapy, or

(iii) the plasma glucose concentration in the fasting blood sample was $\geq$ 7.8 mmol/l and/or the 2-hour blood sample was $\geq$ 11.1 mmol/l.

Impaired glucose tolerance was diagnosed if the plasma glucose concentration was between 7.8 and 11.1 mmol/l in the 2-hour blood sample. The glucose levels used to define diabetes and impaired glucose tolerance were consistent with the WHO criteria for diagnosis of type 2 diabetes and impaired glucose tolerance (WHO 1980).

A positive response to the diabetes question in question 9 alone (i.e. without evidence of insulin or oral therapy or a missing oral glucose tolerance test result) was not considered sufficient evidence of diabetes. This effectively excluded those few subjects who claimed a doctor's diagnosis of diabetes but denied taking any diabetic medication and subsequently underwent a normal oral glucose tolerance test.

3.3.3 Statistical analysis

Information collected from the self-administered smoking questionnaire (Appendix I) was used to classify subjects according to smoking status (current smokers, ex-smokers and never smokers). The current smoking histories were considered sufficiently valid, since stated consumption correlated with mean serum thiocyanate levels (Fowkes et al 1992). Lifetime cigarette smoking was calculated in pack-years (years of smoking multiplied by the average number of packs smoked per day). Lifelong non-smokers received a value of zero, and the square root of pack-years was used to reduce the distortion to the normal distribution resulting from these extreme values.

Other variables, including systolic and diastolic brachial blood pressure, total serum cholesterol, serum high density lipoprotein (HDL) cholesterol and serum triglycerides were available directly from values recorded in the research clinic and the laboratory respectively. Serum low density lipoprotein (LDL) cholesterol was calculated using the formula, LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5 (Friedewald et al 1972), and weight and height measurements were used to calculate body mass index (BMI), according to the formula, BMI = weight (kg)/height (m^2).

Data were analysed on the Edinburgh University mainframe computer (UNIX system) using the SPSS and SAS (Version 6.11) software packages. Variables were checked for normality of distribution and transformed if found to be skewed. Tests for differences in

the age-adjusted mean levels of the risk factors between disease groups were conducted using t-tests.

Logistic regression using the statistical package Proc GENMOD (SAS 1993) was used to calculate the odds ratios of having peripheral arterial disease for a person who had diabetes/impaired glucose tolerance compared to the risk of peripheral arterial disease for a person in the normal glucose tolerance group. Age and sex adjustments were made and then each of the other potentially related factors was individually included in a multivariate model.

Chapter 4

Methods II

Case control study: Plasma insulin and steroid sex hormones as risk factors for peripheral arterial disease

This chapter presents the methods of the case control study, designed to investigate the relationship between endocrine risk factors (plasma insulin and steroid sex hormones) and peripheral arterial disease in male and female cases and controls from the general population.

4.1 Study design and population

All subjects for this case control study were selected from the Edinburgh Artery Study database. Using this database meant that subjects with and without disease were readily identified, but were otherwise randomly selected members of the general population. Cases and controls were selected from subjects attending for clinical examination five years after the initial study examination, and who had not subsequently been notified as dead (through flagging of notes at the UK National Health Service Central Registry or notification from general practitioners or

relatives). Ethical approval was granted for the study by the Lothian Health Board Ethics of Medical Research Sub-Committee for Medicine/Clinical Oncology.

4.2 Sample size

The sample size had to be adequate to detect significant differences between the cases and controls in the main variables of interest. Since data was lacking on possible differences in steroid sex hormone levels between subjects with and without peripheral arterial disease, the sample size was based on predicted differences in plasma insulin levels.

In his study on men with and without peripheral arterial disease, Sloan et al (1970) found a difference in mean plasma insulin levels of 37.1 mU/l between cases and controls, one hour after a standard oral glucose tolerance test. This difference was significant at the 0.1% level. A difference of 1.3 mU/l in fasting levels was not significant.

In a large population-based study, Pyörölä et al (1985), found the following values for serum insulin amongst 982 men aged 35 to 64 years of age;

	Population mean	Standard Deviation
Fasting plasma insulin (mU/l)	7.6	4.7
One-hour plasma insulin (mU/l)	62.4	43.9

The sample size calculation was based on the following formula:

$$\text{number of subjects in each group, } n = \frac{2\sigma^2}{(\mu_2-\mu_1)^2} \times f$$

where $(\mu_2-\mu_1)$ is the difference in mean plasma insulin to be detected

σ is the standard deviation of plasma insulin

f is a constant, depending on the power and significance level required

This constant (f), is equivalent to the term $(\upsilon + \nu)^2$

where υ is the one-sided percentage point of the normal distribution corresponding to 100% - the power, e.g. if power = 90%, (100% - power) = 10% and $\upsilon = 1.28$ ($\upsilon = 1.64$ when power = 95%)

ν is the percentage point of the normal distribution corresponding to the required (two-sided) significance level, e.g. if significance level = 5%, $\nu = 1.96$

With a significance level of 5%, f is equal to 10.5 (power = 90%) or 13 (power = 95%).

Using the standard deviation in one-hour plasma insulin reported by Pyörölä et al (1985), it was estimated that 36 subjects would be required in each group (cases and controls) to detect a difference of 37.1 mU/l or greater in one-hour plasma insulin levels in the present study (power 95%, significance level 5%).

In order to allow for multivariate analysis, it was proposed to increase this number to 45. To investigate the relationship between plasma insulin and disease in both sexes, 45 male cases and 45 female cases, together with equal numbers of age and sex matched controls would be required, giving a final sample size of 90 cases and 90 controls.

4.3 Selection of cases and controls

4.3.1 Definition of cases and controls

In selecting cases from subjects attending the 5-year follow-up examination of the Edinburgh Artery Study population, subjects were defined as a case if they had either;

(i) a history of intermittent claudication according to the WHO intermittent claudication questionnaire, plus an ABPI ≤0.9 in at least one limb, or

(ii) asymptomatic peripheral arterial disease indicated by an ABPI ≤0.85 in at least one limb.

Completion of the WHO intermittent claudication questionaire and measurement of the ankle brachial pressure index in both ankles were the two methods used to assess lower limb arterial disease at the Edinburgh Artery Study five-year follow-up examination. Measurement of the ankle brachial pressure index was performed using the same technique as at baseline. The reactive hyperaemia test which was performed at baseline was not repeated at the five-year follow-up examination, predominantly due

to poor subject compliance (many subjects complained of mild discomfort during the course of this test, and the investigators did not want to jeopardise the continuing participation of these subjects in the study).

In selecting controls from the same population, a subject was defined as a control if they had no evidence of cardiovascular disease, as indicated by all of the following;

(i) no history of intermittent claudication according to the WHO intermittent claudication questionnaire

(ii) an ankle brachial pressure index of 1.0 or greater in both legs

(iii) no history of angina, myocardial infarction or stroke according to the WHO chest pain questionnaire and/or a doctor's diagnosis

(iv) no evidence of myocardial infarction or ischaemia on electrocardiogram

(v) no history of arterial surgery

Evidence of cardiovascular disease was obtained from data collected at baseline, at the five-year follow-up examination and during the intervening five year follow-up period. To obtain details of non-fatal events during follow-up (including myocardial infarction, angina pectoris, stroke and intermittent claudication), information was sought from general practitioners, hospitals and the Information and Services Division of the Scottish Office Home and Health Department. Subjects were also sent an annual questionnaire which included validated questions on cardiovascular history, intermittent claudication (Rose 1962) and angina (Rose 1962). All cardiovascular events and deaths were further investigated using hospital or general

practitioner records. At the follow-up examination, subjects completed a further self-administered medical questionnaire. In addition, a 12-lead electrocardiogram was taken and coded as at baseline (Minnesota coding).

4.3.2 Exclusion criteria

Subjects were excluded from both the case and control groups if they had any of the following;

(i) known diabetes mellitus or newly-diagnosed diabetes according to a fasting plasma glucose ≥ 7.8 mmol/l, or

(ii) use of drugs affecting carbohydrate metabolism (achieved by scrutinising the list of medication taken by eligible cases and controls), or

(iii) women using postmenopausal hormone replacement therapy.

Subjects with diabetes were defined as those taking medication used in the treatment of diabetes (insulin or oral hypoglycaemics), or who had a fasting blood glucose reading of 7.8 mmol/l or greater at the baseline Edinburgh Artery Study examination. Two subjects were excluded subsequently on the basis of their oral glucose tolerance test performed for the purpose of this study.

Women taking hormone replacement therapy included those who listed hormone replacement therapy as one of their 'regular medications' on the Edinburgh Artery

Study medical questionnaire. However, since some women may not have regarded hormone replacement therapy as a medication (and therefore failed to list it), all women who were otherwise eligible for the study were asked directly whether or not they were currently taking hormone replacement therapy. This was done both in the clinic invitation letter (Appendix II) and in person at the clinic (Appendix III).

4.3.3 Matching procedure

Controls were matched to the cases by sex and 5-year age band using the list of eligible cases and controls obtained from the Edinburgh Artery Study database. This list was arranged by sex and 5-year age band (using subject's date of birth), but within each band subjects were listed in random order. Subjects were invited sequentially at a rate of 18 per week to attend one of three morning research clinics, until the required number had been seen. Once a case had agreed to attend, a control was selected from the corresponding sex and 5-year age band. If the control declined the invitation, an alternative random control was substituted from within the same age and sex band as the originally selected control.

4.4 Sample recruitment

All selected cases and controls were sent a letter inviting them to attend for examination (Appendix II). The letter thanked subjects for their recent participation in the Edinburgh Artery Study, explained the purpose of the present study, and outlined

what would be required if they agreed to participate. In particular, it was emphasised that subjects would have to fast for the 12 hours prior to attending for examination. To avoid having to re-contact all the subjects again, and so help to maximise response, each subject was provided with a specific appointment in this initial invitation letter. A telephone number was provided so that the subject could contact the investigator to discuss the study further before deciding whether or not to participate. A map was enclosed, since the clinic was located at a site not previously used in the Edinburgh Artery Study.

Subjects were asked to indicate on a reply slip whether they were able to attend for the specified appointment, whether they would require a different appointment date or whether they were unwilling to participate in the study. Those who refused to participate were not contacted again. Subjects requesting an alternative appointment were contacted by telephone to arrange a suitable date. A second letter confirming the change in appointment was sent to these subjects.

Non-responders to the invitation letter were contacted by telephone, and, if possible persuaded to attend. Those agreeing to attend were sent a letter confirming the agreed appointment. The few subjects who accepted the invitation and then failed to keep their appointment were also contacted by telephone; an alternative appointment was arranged and confirmed by letter.

In all instances where a second letter to either change or confirm an appointment was sent, this contained information stressing the importance of fasting overnight prior to

the examination. This was to remind subjects of the need to fast without having to refer back to their original invitation letter and so minimise the number of subjects who attended without fasting.

4.5 Clinical examination

Clinic procedure: Six subjects were invited to each of three weekly clinics. These were arranged for first thing in the morning because of the requirement to attend after an overnight fast. Usually 4 or 5 subjects agreed to participate per clinic; these went through each stage of the examination simultaneously. As soon as a subject arrived at the clinic, the investigator confirmed that they had fasted overnight (if they had not fasted, an alternative date for the examination was given and the subject sent home). Subjects were also asked whether or not they were diabetic; this was necessary to exclude subjects who had been diagnosed as diabetic very recently and had therefore not yet registered as such on the Edinburgh Artery Study database. Female subjects were also asked to confirm that they were not currently taking hormone replacement therapy. The responses to these questions were noted on the data collection form (Appendix III).

Informed consent: Subjects still found to be eligible for the study had the purpose and format of the study explained to them and they were asked to sign a specially designed consent form (Appendix IV). The consent form was co-signed by the investigator and dated.

Fasting venepuncture: Prior to venepuncture, the investigator checked that the subject was not 'high risk' in terms of possible hepatitis or human immunodeficiency virus (Appendix V). A 30 ml venous blood sample was then taken with the subject in a recumbent position. Any practical problems with the venepuncture (such as very slow blood flow into the syringe) were noted on the venepuncture form, together with the amount of blood taken. This was done in order to identify potential reasons for any samples found subsequently to be grossly abnormal or missing. The blood sample was divided into four pre-labelled collecting tubes as follows;

- 2.5 ml in a fluoride oxalate tube for measurement of blood glucose
- 10 ml in a lithium heparin tube for measurement of plasma insulin
- 10 ml in a plain tube for measurement of total serum cholesterol, high density lipoprotein cholesterol and triglycerides
- 8 ml in a second lithium heparin tube for measurement of total plasma testosterone, sex hormone-binding globulin, oestrone and oestradiol

Apart from the glucose sample, more blood than was required for the stated assays was collected in each tube. This meant that there would be one serum sample and two plasma samples per patient available for freezing and storage, allowing measurement of other potential risk factors at a later date.

Oral glucose tolerance test (OGTT): Following venepuncture, all cases and controls underwent a standard 75g oral glucose tolerance test. This was performed in ambulatory conditions and without dietary preparation using 389 ml of Lucozade

Sparkling Glucose Drink (equivalent to the glucose load of 75g recommended by the World Health Organisation for the OGTT in adults). A further 10 ml venous blood sample for plasma insulin and blood glucose determination was taken 60 minutes after glucose administration. The sample was divided between a 2.5 ml fluoride oxalate tube (for blood glucose measurement) and a 10ml lithium heparin tube (for plasma insulin measurement).

Waist hip ratio measurement: During the hour between glucose administration and post-glucose venepuncture, each subject underwent measurement of their waist and hip circumferences. These were measured to the nearest centimetre with the subject standing and breathing normally. Hip measurements were made at the level of the iliac crest and all measurements were made by a single observer. The waist hip ratio was calculated as the simple ratio of waist to hip circumference.

Data on smoking history, blood pressure, height and weight was taken from the Edinburgh Artery Study 5-year follow-up examination, where these factors were recorded by the same methods as in the cross-sectional survey. This included completion of a self-administered questionnaire with validated questions on smoking, measurement of right brachial systolic and diastolic blood pressures after 5 minutes rest using a random zero sphygmomanometer, and standard height and weight measurements.

4.6 Blood processing

Plasma insulin: The fasting and post-glucose blood samples for measurement of plasma insulin were centrifuged at 4°C and 3000 r.p.m for 20 minutes within 15 minutes of collection. 2 mls of the supernatant plasma was pipetted into labelled microtubes and immediately frozen at -40°C. Samples were stored at -40°C for a maximum of six months before being delivered in batches to the Metabolic Unit, Clinical Biochemistry Department, Western General Hospital, Edinburgh for analysis. Any remaining plasma was pipetted into labelled microtubes and frozen at -40°C for storage.

Blood glucose: The fasting and post-glucose blood samples for measurement of blood glucose were centrifuged at 4°C and 3000 r.p.m for 20 minutes. The resultant supernatant (approximately 1.5 ml) was pipetted into labelled microtubes and immediately frozen at -40°C. Samples were stored at -40°C before being delivered in batches to the Metabolic Unit at the Western General Hospital, together with the samples for plasma insulin measurement.

Total plasma testosterone, sex hormone-binding globulin, oestrone and oestradiol: The 8 ml blood sample for measurement of steroid sex hormones was centrifuged at 4°C and 3000 r.p.m for 20 minutes, usually within 15 minutes of collection. 2 mls of the supernatant plasma was pipetted into a labelled microtube and immediately frozen at -40°C. The samples were stored at -40°C for a maximum of six months before being delivered to the Reproductive Medicine Centre, University of Edinburgh for analysis.

Any remaining plasma was pipetted into a labelled microtube and frozen at -40°C for storage.

Serum cholesterol, high density lipoprotein cholesterol and triglycerides: The 10 ml fasting blood sample for measurement of serum lipids was left to stand for approximately one hour until clotting had taken place. The sample was then centrifuged at 4°C and 3000 r.p.m. for 20 minutes. 2 mls of the supernatant serum was pipetted into a labelled microtube and frozen at -40°C until delivery to the Clinical Biochemistry Department, Western General Hospital, Edinburgh for analysis. The remaining supernatant was pipetted into a labelled microtube and frozen at -40°C for storage.

4.7 Quality control

To check the quality of laboratory assays, duplicate samples were taken periodically throughout the course of the study. This was done on subjects where the amount of plasma or serum obtained was sufficient to produce two samples rather than the usual one. In each case, the second sample was assigned a 'dummy' subject number. Only the investigator was aware of which subject this number referred to. A total of 10 samples were duplicated in this way for each of the factors measured. Each laboratory also employed their own quality control measures using 'standard' assay concentrations.

4.8 Laboratory assays

Plasma insulin concentration was measured using a microparticle enzyme immunoassay on an IMx analyser (Abbott Laboratories Limited, Maidenhead, UK). The results of this assay were reported in the conventional units of mU/l (these units are equivalent to those of μU/ml reported in some previous studies). The assay measurement range was 1-300 mU/l, with a sensitivity of 1 mU/l. Reproducibility of the method was assessed in the laboratory using 3 quality control specimens. Intra- and inter-assay coefficients of variation at an insulin concentration of 8.3 mU/l were 4.0% and 4.5% respectively. The IMx assay showed no cross-reactivity with pro-insulin (<0.005%), potentially giving lower values than observed with other less specific immunoassays, and no detectable cross-reactivity with C-peptide or glucagon. Although cross-reactivity with bovine and porcine insulins has been observed with the IMx insulin assay, this was not a problem in the present study since none of the subjects were on insulin therapy.

Insulin resistance was calculated as an index according to the equation, insulin resistance = (fasting glucose x fasting insulin)/22.5 (Matthews et al 1985).

Blood glucose concentration was measured by a timed end-point enzymatic method employing hexokinase and glucose-6-phosphate dehydrogenase using a Beckman Synchron CX5 multichannel analyser (Beckman Instruments (UK) Ltd, High Wycombe, Bucks, UK). The assay analytical range was 0.3-38.8 mmol/l, with a sensitivity of 0.3 mmol/l. The inter-assay coefficient of variation was 0.8%. This is one of the most specific methods for measurement of glucose.

Plasma steroid sex hormones: Oestradiol was measured by radioimmunoassay using sheep anti-oestradiol antibody. Oestrone was measured by ELISA using rabbit anti-estrone-3-glucuronide-BSA antibody. Sex hormone-binding globulin and total testosterone were measured by radioimmunoassay using commercial kits (Orion Diagnostica and Medgenix respectively). All samples were assayed in duplicate and the sample was assayed again if the values for the individual duplicates differed by more than 5%. Inter- and intra-assay coefficients of variation were $\leq$ 9.2% for estradiol, $\leq$ 9.9% for estrone, $\leq$ 9.3% for sex hormone-binding globulin and $\leq$ 11.7% for total testosterone. For subjects whose hormone levels were found to be below the level of sensitivity of the total testosterone assay (0.18 nmol/l) and the estradiol assay (30 pmol/l), these values were used in the analysis. Free testosterone was calculated using the Free Androgen Index according to the equation, free testosterone (pmol/l) = [1000 x total testosterone (nmol/l)] / sex hormone binding globulin (nmol/l) (Nanjee and Wheeler 1985).

Total serum cholesterol was measured by an enzymatically-linked reaction using cholesterol ester hydrolase, cholesterol oxidase and peroxidase using a E750C dry chemistry analyser (Ortho Clinical Diagnostics, Hemel Hempstead, UK). The analytical range of this assay was 1.29-8.40 mmol/l; above 8.4 mmol/l, samples were re-run on dilution, so there was no upper limit. Within-batch precision was between 0.92% and 1.17% and between-batch precision was between 1.4% and 2.6% (recorded at mean concentrations of 2.74, 5.46 and 6.83 mmol/l).

Serum triglycerides were measured by an enzymatic method using linked reactions and lipase, glycerol kinase, glycerophosphate oxidase and peroxidase using a E750 C dry chemical analyser as for total cholesterol. The analytical range of this assay was 0.11-5.90 mmol/l; for values above this, samples were re-analysed on dilution. Within-batch precision was between 0.88% and 1.32% and between-batch precision was between 1.6% and 1.9% (measured at mean concentrations of 0.99, 2.62 and 4.99 mmol/l).

Serum high density lipoprotein (HDL) cholesterol was measured on a Cobas Mira Plus analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). All other cholesterol-containing lipoproteins were precipitated by $MgCl_2$/Phosphotungstic acid leaving HDL cholesterol in solution. After centrifuging at 6000 rpm for 5 minutes, cholesterol was then measured in the supernatant enzymatically using cholesterol esterase, cholesterol oxidase and peroxidase. The analytical range of this assay was 0.2-5.0 mmol/l. Between-batch precision was 4.6% and 3.4% (measured at mean concentrations of 0.73 and 1.99 mmol/l respectively).

Low density lipoprotein (LDL) cholesterol was calculated using the formula, LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5 (Friedewald et al 1972).

4.9 Statistical analysis

Mean levels of risk factors were compared in study cases and controls and the significance of differences were assessed by t-test, using the statistical package SPSS-X. For haematologic factors with skewed distributions, levels were log transformed before statistical analysis. Mean levels were then anti-logged to give the more readily understandable geometric mean in the original units (confidence intervals were also transformed back to arithmetic units). Cigarette smoking was calculated in pack-years (years of smoking multiplied by the average number of packs smoked per day) as a measure of lifetime smoking. Lifelong non-smokers received a value of zero, and the square root of pack-years was used to reduce the distortion to the normal distribution resulting from these extreme values.

Spearman's rank correlation coefficients were calculated to examine associations between one-hour insulin levels and steroid sex hormones and the other cardiovascular risk factors. These were calculated separately for cases and controls because they represented two distinct groups. Multiple logistic regression was used to investigate the independence of the relationship between risk factors and disease after controlling for age and other cardiovascular risk factors. The odds of peripheral arterial disease associated with a one log unit increase in one-hour insulin concentration was estimated from the logistic regression coefficients obtained by the statistical package BMDP, and expressed as an odds ratio. The odds of disease for a one unit increase in each of the steroid sex hormone was also calculated for men and women separately. A one unit increase on a log scale was used for sex hormone-binding globulin and serum oestrone,

due to their skewed distributions. For the other hormones, the unit increase was one standard deviation.

To investigate the relationship between smoking status and plasma insulin levels, geometric means and transformed confidence intervals of one-hour insulin were calculated for never-smokers and ever-smokers (current and ex-smokers). Multiple linear regression was performed (with smoking as the independent variable and one-hour insulin as the dependent variable) to determine the independence of the relationship after controlling for the other cardiovascular risk factors. The following independent variables had forced entry into the final model: presence or absence of peripheral arterial disease, sex, age and smoking status.

Chapter 5

Results I

Edinburgh Artery Study: Relationship between diabetes and cardiovascular risk factors in the development of peripheral arterial disease

This chapter describes the results of analysis on the Edinburgh Artery Study cross-sectional data to investigate the relationship between diabetes, cardiovascular risk factors and peripheral arterial disease.

5.1 Characteristics of study population

5.1.1 Characteristics of responders and non-responders

During recruitment for the Edinburgh Artery Study, invitations were sent to 2709 subjects of whom 1592 (809 men and 783 women) ultimately participated in the study, a crude response rate of 59%. Follow-up of the random sample of non-responders showed that 19% had moved and 3% were dead or in hospital. Extrapolation of these results to the whole sample suggested that the response rate of those receiving an invitation was 65%.

Previous analysis undertaken by the study director indicated that the responders were reasonably typical of the target population (Fowkes et al 1991). Thus the crude response rate did not differ substantially by age or sex, although there was slight under-representation of women aged 70-74 years and males aged 55-59 years who comprised 21.3% and 22.5% (instead of 25.0%) of women and men respectively. The social class distribution of responders was similar to that of Edinburgh adult residents in the 1981 census except that the responders contained fewer subjects from social classes IV and V (13% compared to 19%). The crude response rate varied between the ten general practices, from 47% to 71%, with the lower response rates occurring in practices serving deprived areas, thus also suggesting a slight under-representation of lower social class.

5.1.2 Characteristics of subjects with diabetes or impaired glucose tolerance

The diabetic status of all subjects attending the Edinburgh Artery Study is given in Table 5.1. Two hundred and eighty eight (288) subjects (18.7%) had diabetes (n = 91) or impaired glucose tolerance (n = 197) compared with 1253 subjects with normal glucose tolerance (51 subjects could not be classified due to missing data). Of the 91 subjects with diabetes, 8 (8.8%) were insulin treated, 18 (19.8%) were on oral treatments, and 17 (18.7%) did not admit to taking any treatment other than diet modification: the remainder were previously undiagnosed diabetics who were detected using the oral glucose tolerance test (52.7%, n=48).

5.1.3 Characteristics of subjects with peripheral arterial disease

The characteristics of subjects with peripheral arterial disease are given in Table 5.2. Of the 1541 subjects who could be classified by diabetic status, a total of 171 (11.1%) had peripheral arterial disease according to the study criteria. Of these, 69 (40.4%) had symptomatic disease (a positive WHO intermittent claudication questionnaire) and 102 (59.6%) had major asymptomatic disease. Fifty one (29.8%) were classified as asymptomatic disease according to an ankle brachial pressure index of 0.9 or less and a drop in ankle systolic blood pressure during the reactive hyperaemia test of more than 20%. A further 35 (20.5%) had asymptomatic disease with an ankle brachial pressure index of 0.7 or less alone and 16 (9.4%) had a hyperaemic drop of more than 35% alone.

A total of 1051 subjects were entered into the 'healthy group'. These were subjects with none of the criteria for the 'disease group' plus an ankle brachial pressure index greater than 0.9 and a hyperaemic pressure reduction of less than 20%.

5.2 Univariate analysis

5.2.1 Prevalence of peripheral arterial disease according to diabetic status

A total of 1222 subjects could be classified according to diabetic status and had either

symptomatic or major asymptomatic peripheral arterial disease (n=171) or were included in the 'healthy' group (n=1051). Sixty seven (67) of these subjects had diabetes, 151 had impaired glucose tolerance (IGT) and 1004 subjects had normal glucose tolerance.

Figure 5.1a gives the prevalence of peripheral arterial disease (symptomatic and major asymptomatic disease) by diabetic status. Peripheral arterial disease was more common in subjects with diabetes (22.4%, n=15) and with impaired glucose tolerance (19.9%, n=30) than in those with normal glucose tolerance (12.5%, n=126, $p \leq 0.05$). This difference persisted, although to a slightly lesser extent, after age-adjustment (Figure 5.1b). The prevalence of disease was not significantly different between subjects with diabetes and those with impaired glucose tolerance (22.4% vs 19.9%, p=0.7), and these groups were combined to increase the power of the study. Just over a half of the total prevalence of peripheral arterial disease in subjects with diabetes or impaired glucose tolerance (20.6%, n=45) was due to asymptomatic disease (11.5%, n=25). In the group with normal glucose tolerance, slightly more of the total prevalence of peripheral arterial disease was due to asymptomatic disease (7.7%, n=78 compared with 4.8%, n=48 for symptomatic disease).

5.2.2 Risk factor characteristics of 'disease group' compared with 'healthy group' in diabetic/IGT study population

Within the diabetic/impaired glucose tolerant study population, mean levels of risk factors were compared between subjects with peripheral arterial disease (disease group)

and those without disease (healthy group). There was no significant difference in the sex distribution between the two groups (43% of the disease group and 58% of the healthy group were men, $p>0.1$). However, subjects with peripheral arterial disease were older (mean age 68.8 ±0.8 years) than those in the healthy group (mean age 65.1 ±0.4 years, $p\leq0.001$). Mean levels of the other risk factors were therefore presented (and analysed statistically) following adjustment for age (Table 5.3).

More subjects with peripheral arterial disease were current or ex-smokers (74.1%) than in the healthy group (48.1%, $p\leq0.05$) and lifetime smoking (measured as square root pack-years to normalise the skewed distribution of pack-years) was significantly higher in the disease group (mean 4.21 ±0.49 vs 2.38 ±0.23, $p\leq0.001$).

Systolic blood pressure, diastolic blood pressure and body mass index were all normally distributed within the study population and so did not require transformation prior to analysis. Age adjusted mean systolic blood pressure was significantly higher in the disease group (161.8 ±3.7mmHg) than in the healthy group (150.4 ±1.8 mmHg, $p\leq0.01$). However, there was no significant difference between the two groups in mean diastolic blood pressure, or in mean body mass index ($p>0.1$).

All of the serum lipids were found to be normally distributed in the study population except for serum tryglycerides which was positively skewed. Values for serum triglycerides were therefore log transformed before statistical analysis and geometric means presented. Mean levels of total serum cholesterol and serum low density lipoprotein cholesterol appeared slightly higher in the disease group than in the healthy

group (mean LDL cholesterol 5.61 ±0.20 mmol/l vs 5.25 ±0.10 mmol/l), but these differences were not statistically significant (p=0.12 for LDL cholesterol and p=0.15 for total cholesterol). The difference in mean serum high density lipoprotein cholesterol was also non-significant (1.29 ±0.06 mmol/l in disease group vs 1.38 ±0.03 mmol/l in healthy group, p=0.19). However, mean serum triglycerides *were* significantly higher in the disease group (0.69 ±0.08 mmol/l) than in the healthy group (0.46 ±0.04 mmol/l, $p \leq 0.01$)

5.2.3 Risk factor characteristics of 'disease group' compared with 'healthy group' in normal glucose tolerant study population

Levels of risk factors were then compared between the disease group and the healthy group in subjects with normal glucose tolerance. Again, there was no significant difference in the sex distribution between the two groups, but subjects with peripheral arterial disease were found to be older (mean age 66.9 ±0.5 years) than those in the healthy group (mean age 63.9 ±0.2 years, $p \leq 0.001$).

Mean levels of the other risk factors following age-adjustment are given in Table 5.4. More subjects with peripheral arterial disease were current or ex-smokers (83.5%) than in the healthy group (61.6%, $p \leq 0.01$), and lifetime smoking (square root pack-years), was significantly higher in the disease group (mean 4.67 ±0.25 vs 2.81 ±0.09, $p \leq 0.001$). As in the diabetic/IGT study population, age adjusted mean systolic blood pressure was significantly higher in the disease group (147.8 ±1.9 mmHg) than in the healthy group

(138.7 ±0.7 mmHg ,p≤0.001), but there was no significant difference between the two groups in mean diastolic blood pressure, or in mean body mass index (p>0.1).

Subjects with peripheral arterial disease had significantly higher mean levels of total serum cholesterol and serum low density lipoprotein cholesterol compared with those without disease (mean LDL cholesterol 5.62 ±0.11 mmol/l vs 5.14 ±0.04 mmol/l, p≤0.001). They also had lower mean serum high density lipoprotein cholesterol (1.38 ±0.04 mmol/l vs 1.47 ±0.01 mmol/l, p≤0.05). As in the diabetic/IGT study population, mean serum triglycerides were significantly higher in the disease group (0.46 ±0.04 mmol/l) than in the healthy group (0.27 ±0.02 mmol/l, p≤0.001)

5.2.4 Risk factor characteristics of subjects with diabetes or IGT compared with normal glucose tolerant subjects

Mean risk factor levels were then compared between subjects with diabetes or impaired glucose tolerance and those with normal glucose tolerance. This was done separately for subjects with and without peripheral arterial disease since the presence of peripheral arterial disease could confound any relationship between diabetes/IGT and risk factor levels. In both subjects with and without peripheral arterial disease, the diabetic/IGT populations were older (p≤0.05) than the normal glucose tolerant subjects, so mean levels of the other cardiovascular risk factors were age-adjusted.

In subjects with peripheral arterial disease (Table 5.5), the diabetic/IGT population had higher mean levels of systolic blood pressure (161.8 ±3.7 mmHg vs 147.8 ±1.9 mmHg,

p≤0.01) and serum triglycerides (0.69 ±0.08 mmol/l vs 0.46 ±0.04 mmol/l, p≤0.05), compared with the normal glucose tolerant population. These risk factors were also raised in the diabetic/IGT population in subjects without peripheral arterial disease (Table 5.6). In addition, in subjects without peripheral arterial disease, those with diabetes or impaired glucose tolerance had raised mean diastolic blood pressure (78.9 ±0.9 mmHg vs 76.1 ±0.4 mmHg, p≤0.01) and body mass index (26.8 ±0.3 kg/m2 vs 25.0 ±0.1 kg/m2, p≤0.001) and reduced mean serum high density lipoprotein cholesterol (1.38 ±0.03 mmol/l vs 1.47 ±0.01 mmol/l, p≤0.01). However, subjects with diabetes or impaired glucose tolerance smoked less than normal glucose tolerant subjects; only 48.1% were current or ex smokers compared with 61.6% of subjects with normal glucose tolerance (p≤0.001).

5.3 Multivariate analysis

Multiple logistic regression was used to examine the relationship between diabetes/impaired glucose tolerance and risk of peripheral arterial disease, before and after adjustment for the other cardiovascular risk factors. The presence of diabetes or impaired glucose tolerance was associated with a significant increase in the risk of peripheral arterial disease (odds ratio for peripheral arterial disease in the diabetic/IGT group compared with the normal glucose tolerant group was 1.64; 95% CI 1.17, 2.31 p≤0.01). After adjusting for age and sex, subjects with diabetes or impaired glucose tolerance still had a higher risk of disease (odds ratio 1.45; 95% CI 1.03, 2.04, p≤0.05).

The results of further adjustment for each of the cardiovascular risk factors in turn is shown in Table 5.7. Adjustment for smoking increased the risk of disease in subjects with diabetes or impaired glucose tolerance slightly (odds ratio 1.65; 95% CI 1.16, 2.34). This was not surprising given that smoking was a significant risk factor for peripheral arterial disease and that levels of smoking were lower in the diabetic/IGT population.

Adjustment for diastolic blood pressure and body mass index had little effect on the risk of disease. However, following adjustment for systolic blood pressure, subjects with diabetes or impaired glucose tolerance no longer had a significantly higher risk of peripheral arterial disease than those with normal glucose tolerance (odds ratio 1.22, 95% CI 0.85, 1.73, p=0.3)

Adjustment for total serum cholesterol and serum low density lipoprotein cholesterol, also had little effect on the risk of disease. However, following adjustment for serum triglycerides, the odds ratio fell to a non-significant value of 1.26 (95% CI 0.89, 1.79, p=0.2). After adjusting for high density lipoprotein cholesterol, subjects with diabetes or impaired glucose tolerance still had higher levels of peripheral arterial disease, but the odds ratio was of borderline significance (odds ratio 1.37; 95% CI 0.97, 1.94, p=0.08).

Simultaneous adjustment for both systolic blood pressure and serum triglycerides reduced the risk of disease among subjects with diabetics or impaired glucose tolerance to 1.11 (95% CI 0.78, 1.58, p=0.6).

In summary, in subjects with diabetes or IGT, mean levels of smoking, systolic blood pressure and serum triglycerides were significantly higher in subjects with peripheral arterial disease than in those without disease ($p \leq 0.05$). In general, levels of cardiovascular risk factors were higher in subjects with diabetes or IGT compared with normal glucose tolerant subjects, including systolic blood pressure and serum triglycerides (but not smoking). In multivariate analysis, subjects with diabetes or IGT no longer had a significantly higher risk of peripheral arterial disease after adjusting separately for systolic blood pressure (odds ratio 1.22, 95% CI 0.85, 1.73) and serum triglycerides (odds ratio 1.26, 95% CI 0.89, 1.79). Simultaneous adjustment for both systolic blood pressure and serum triglycerides reduced the risk further to 1.11 (95% CI 0.78, 1.58).

Table 5.1. Classification of all subjects attending Edinburgh Artery Study according to diabetic status

Subject group	*n*	*%*
Diabetes	91	5.9
Insulin treated	8 (8.8%)	
Oral medication	18 (19.8%)	
Diet modification	17 (18.7%)	
Newly diagnosed on OGTT	48 (52.7%)	
Impaired glucose tolerance	197	12.8
Normal glucose tolerance	1253	81.3
TOTAL	1541	100

(51 subjects un-classified due to missing data)

OGTT, oral glucose tolerance test

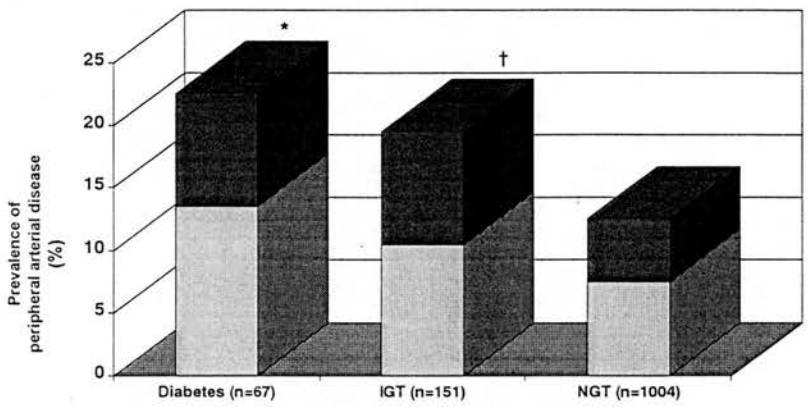
Table 5.2. Classification of subjects by measures of peripheral arterial disease (in those 1541 subjects who could be classified according to diabetic status)

Study Group	*n*	*%*
Peripheral arterial disease		
Symptomatic disease		
Intermittent claudication	69	40.4
Major asymptomatic disease		
ABPI ≤ 0.9 and Hyperaemia > 20%	51	29.8
ABPI ≤ 0.7 alone	35	20.5
Hyperaemia > 35% alone	16	9.4
Total	171	100
'Healthy'	1051	
TOTAL	1222	

(51 subjects with missing data on diabetic status and 319 subjects with 'minor' asymptomatic PAD omitted)

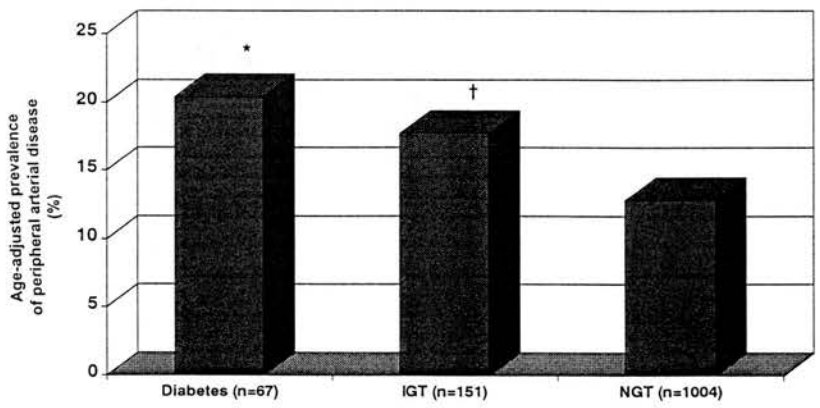
Intermittent claudication Grades 1 (n=23), 2 (n=31) and probable (n=15)
Hyperaemia - hyperaemic reduction in ankle pressure after occlusion
ABPI - ankle brachial pressure index
'Healthy' - none of the criteria for symptomatic or major asymptomatic peripheral arterial disease, plus an ABPI >0.9 and a hyperaemic pressure reduction <20%

Figure 5.1a Prevalence of peripheral arterial disease, categorised according to major asymptomatic disease and intermittent claudication in subjects with diabetes, impaired glucose tolerance (IGT) and normal glucose tolerance (NGT)



Difference in prevalence from NGT group: * p≤0.05, † p≤0.01

Figure 5.1b Age-adjusted prevalence of peripheral arterial disease in subjects with diabetes, impaired glucose tolerance (IGT) and normal glucose tolerance (NGT)



Difference in prevalence from NGT group: * p≤0.05, † p=0.07

Table 5.3 Age, sex and age-adjusted mean levels of risk factors in subjects with diabetes or impaired glucose tolerance, with and without peripheral arterial disease

Risk factor	*Mean (or %), SE*		*p-value*
	PAD (n=45)	*No PAD (n=173)*	
Age, years	68.8 (0.8)	65.1 (0.4)	≤0.001
Sex, % male	43.1	58.4	NS
Smoking			
% current/ex smokers	74.1	48.1	≤0.05
√pack-years	4.21 (0.49)	2.38 (0.23)	≤0.001
Blood pressure			
Systolic blood pressure, mmHg	161.8 (3.7)	150.4 (1.8)	≤0.01
Diastolic blood pressure, mmHg	80.5 (1.9)	78.9 (0.9)	NS
Body Mass Index, kg/m^2	27.0 (0.7)	26.8 (0.3)	NS
Lipids			
Total cholesterol, mmol/l	7.35 (0.22)	6.99 (0.11)	NS
LDL cholesterol, mmol/l	5.61 (0.20)	5.25 (0.10)	NS
HDL cholesterol, mmol/l	1.29 (0.06)	1.38 (0.03)	NS
Triglycerides, ln mmol/l	0.69 (0.08)	0.46 (0.04)	≤0.01

NS, not significant ($p>0.05$); SE, standard error; PAD, peripheral arterial disease
LDL, low density lipoprotein; HDL, high density lipoprotein

Table 5.4 Age, sex and age-adjusted mean levels of risk factors in normal glucose tolerant subjects, with and without peripheral arterial disease

Risk factor	*Mean (or %), SE*		*p-value*
	PAD (n=126)	*No PAD (n=878)*	
Age, years	66.9 (0.5)	63.9 (0.2)	≤0.001
Sex, % male	50.4	52.7	NS
Smoking			
% current/ex smokers	83.5	61.6	≤0.01
√ pack-years	4.67 (0.25)	2.81 (0.09)	≤0.001
Blood pressure			
Systolic blood pressure, mmHg	147.8 (1.9)	138.7 (0.7)	≤0.001
Diastolic blood pressure, mmHg	77.5 (1.1)	76.1 (0.4)	NS
Body Mass Index, kg/m^2	25.2 (0.3)	25.0 (0.1)	NS
Lipids			
Total cholesterol, mmol/l	7.32 (0.12)	6.89 (0.04)	≤0.001
LDL cholesterol, mmol/l	5.62 (0.11)	5.14 (0.04)	≤0.001
HDL cholesterol, mmol/l	1.38 (0.04)	1.47 (0.01)	≤0.05
Triglycerides, ln mmol/l	0.46 (0.04)	0.27 (0.02)	≤0.001

NS, not significant ($p>0.05$); SE, standard error; PAD, peripheral arterial disease
LDL, low density lipoprotein; HDL, high density lipoprotein

Table 5.5 Age, sex and age-adjusted mean levels of risk factors in diabetic/impaired glucose tolerant and normal glucose tolerant subjects with peripheral arterial disease

	Mean (or %), SE		
Risk factor	***Diabetic (n=45)***	***NGT (n=126)***	***p-value***
Age, years	68.8 (0.8)	66.9 (0.5)	≤0.05
Sex, % male	43.1	50.4	NS
Smoking			
% current/ex smokers	74.1	83.5	NS
√ pack-years	4.21 (0.49)	4.67 (0.25)	NS
Blood pressure			
Systolic blood pressure, mmHg	161.8 (3.7)	147.8 (1.9)	≤0.01
Diastolic blood pressure, mmHg	80.5 (1.9)	77.5 (1.1)	NS
Body Mass Index, kg/m^2	27.0 (0.7)	25.2 (0.3)	NS
Lipids			
Total cholesterol, mmol/l	7.35 (0.22)	7.32 (0.12)	NS
LDL cholesterol, mmol/l	5.61 (0.20)	5.62 (0.11)	NS
HDL cholesterol, mmol/l	1.29 (0.06)	1.38 (0.04)	NS
Triglycerides, ln mmol/l	0.69 (0.08)	0.46 (0.04)	≤0.05

NS, not significant ($p>0.05$); SE, standard error; NGT, normal glucose tolerance
LDL, low density lipoprotein; HDL, high density lipoprotein

Table 5.6. Age, sex and age-adjusted mean levels of risk factors in diabetic/impaired glucose tolerant and normal glucose tolerant subjects without peripheral arterial disease

Risk factor	*Mean (or %), SE*		*p-value*
	Diabetic (n=173)	*NGT (n=878)*	
Age, years	65.1 (0.4)	63.9 (0.2)	≤0.05
Sex, % male	58.4	52.7	NS
Smoking			
% current/ex smokers	48.1	61.6	≤0.001
√ pack-years	2.38 (0.23)	2.81 (0.09)	0.07
Blood pressure			
Systolic blood pressure, mmHg	150.4 (1.8)	138.7 (0.7)	≤0.001
Diastolic blood pressure, mmHg	78.9 (0.9)	76.1 (0.4)	≤0.01
Body Mass Index, kg/m^2	26.8 (0.3)	25.0 (0.1)	≤0.001
Lipids			
Total cholesterol, mmol/l	6.99 (0.11)	6.89 (0.04)	NS
LDL cholesterol, mmol/l	5.25 (0.10)	5.14 (0.04)	NS
HDL cholesterol, mmol/l	1.38 (0.03)	1.47 (0.01)	≤0.01
Triglycerides, ln mmol/l	0.46 (0.04)	0.27 (0.02)	≤0.001

NS, not significant (p>0.05); SE, standard error; NGT, normal glucose tolerance
LDL, low density lipoprotein; HDL, high density lipoprotein

Table 5.7. Odds of peripheral arterial disease according to diabetic status (diabetes/IGT versus normal glucose tolerance), before and after adjustment for each risk factor

Risk factors adjusted for (in addition to age and sex)	*Odds Ratio*	*95% Confidence Interval*	*p-value*
-	1.64	(1.17, 2.31)	≤0.01
Sex, age	1.45	(1.03, 2.04)	≤0.05
Smoking status			
Current/ex/never*	1.65	(1.16, 2.34)	≤0.01
Blood pressure			
Systolic blood pressure, mmHg	1.22	(0.85, 1.73)	0.3
Diastolic blood pressure, mmHg	1.42	(1.01, 2.01)	≤0.05
Body Mass Index, kg/m^2	1.42	(1.00, 2.01)	≤0.05
Serum lipids			
Total cholesterol, mmol/l	1.42	(1.01, 2.01)	≤0.05
LDL cholesterol, mmol/l	1.43	(1.01, 2.02)	≤0.05
HDL cholesterol, mmol/l	1.37	(0.97, 1.94)	0.08
Triglyceride, ln mmol/l	1.26	(0.89, 1.79)	0.2
Multiple adjustment			
Systolic BP and triglycerides †	1.11	(0.78, 1.58)	0.6

*smoking status (current, ex, never) used as categorical variable
†simultaneous adjustment for systolic blood pressure and triglycerides (log)
LDL, low density lipoprotein; HDL, high density lipoprotein; BP, blood pressure

Chapter 6

Results II

Case control study: Plasma insulin and steroid sex hormones as risk factors for peripheral arterial disease

This chapter describes the findings of the case control study. It is divided into three sections; (i) general characteristics of the study population, (ii) plasma insulin levels in cases and controls, and (iii) plasma steroid sex hormone levels in male and female cases and controls.

6.1 Characteristics of study population

6.1.1 Characteristics of responders and non-responders

Subjects for the case control study were selected from the 1156 subjects attending the Edinburgh Artery Study 5-year follow-up examination. By this stage, 203 of the original 1592 subjects participating in the baseline cross-sectional survey had died, 131 completed a questionnaire but did not attend for examination and the remainder could not be contacted at home or were unwilling or unable, due to illness, to continue with the study. The response rate for clinic attendance amongst those still living was

82.3% and examination of the characteristics of attenders compared with the total study population eligible to attend for examination showed no significant difference in terms of age, sex or social class distribution (Table 6.1).

Among the 1156 subjects who attended for follow-up, a total of 128 cases and 290 controls were initially identified as eligible for the case control study. The percentage of eligible subjects varied according to 5-year age band (Table 6.2); in general, more cases and fewer controls were eligible from successive age bands (reflecting the increasing prevalence of arterial disease with age).

All fifty four (54) male cases who were identified as eligible for the study were invited for examination. However, of these, two were found to have died and one had been diagnosed as diabetic since the Edinburgh Artery Study five-year follow-up examination, and were therefore ineligible. Of the remaining 51 cases, 40 attended (response rate 78%). Reasons for non-attendance were refusal (n=9), inability to contact subject by letter or telephone (n=1) and illness (n=1).

Sixty seven (67) of 74 female cases who were identified as eligible for the study were invited for examination. The eligible cases who were not invited were all in the oldest (75-80 year) age group; the number of cases who could be invited from this age group was limited by the number of controls available in the corresponding age band (only 19 eligible controls compared with 27 eligible cases). Of the 67 cases invited, one was subsequently found to have had a stroke since the five-year follow-up examination, and was therefore ineligible. Of the remaining 66 cases, 43 attended (response rate 65%).

Reasons for non-attendance were refusal (n=18) and inability to contact subject by letter or telephone (n=5).

A total of 149 subjects were identified who met the criteria for a male control, of which 56 were invited for examination. However, three subjects were subsequently found to be ineligible because of a recent stroke (n=2) or use of steroid medication (n=1). Of the remaining 53 invited controls, 41 attended (response rate 77%). Reasons for non-attendance were refusal (n=8), inability to contact subject (n=3) and illness (n=1).

A total of 141 subjects were identified who met the criteria for a female control, of which 59 were invited for examination. However, one subject was subsequently found to be ineligible because they were using hormone replacement therapy. Of the remaining 58 invited controls, 47 attended (response rate 81%). Reasons for non-attendance were refusal (n=9), inability to contact subject (n=1) and illness (n=1).

Although a control subject was only invited once a case had agreed to attend, more controls than cases ultimately attended for examination (3 men and 4 women). This was because, on several occasions, the case withdrew from the study or was found to be ineligible *after* their matched control had been invited. However, even with these controls included, there was no significant difference in the age or sex distributions of the case and control groups, and so all subjects were retained for the purposes of the analysis.

The final study population consisted of 83 cases and 88 controls. The response rates for cases and controls are given by sex and 5-year age-band in Table 6.3. In general, response rates were higher in the younger age groups, especially in women. However, there was no significant difference in the age, sex or social class distribution between case responders and the total sample of cases eligible for inclusion in the study. There was also no significant difference between these groups in mean ankle brachial pressure index, nor in the prevalence of intermittent claudication (Table 6.4).

6.1.2 Disease characteristics of cases

The disease characteristics of the selected cases are given in Table 6.5. Subjects were defined as a case if they had either a history of intermittent claudication plus an ABPI ≤ 0.9, or an ABPI ≤ 0.85 alone. Of the 83 cases, 26 (31.3%) suffered from intermittent claudication and had an ankle brachial pressure (ABPI) of 0.9 or less; the remainder were asymptomatic, with an ABPI of 0.85 or less. A similar percentage of male and female cases were symptomatic (30.0% and 32.6% respectively).

As expected, mean ankle brachial pressure index was substantially lower in cases (0.71) than in controls (1.12; $p \leq 0.001$). A similar difference in mean ABPI between cases and controls was found for both men (0.70 vs 1.14; $p \leq 0.001$) and women (0.72 vs 1.10; $p \leq 0.001$).

Twenty cases (24.1%) also had evidence of angina and a smaller percentage had evidence of previous myocardial infarction (16.9%) or stroke (3.6%). The controls had

no evidence of intermittent claudication, angina, myocardial infarction or stroke, indicating that they had been correctly selected according to the control criteria.

6.1.3 Comparability of cases and controls

As expected from the matching, age and sex distributions were almost identical in cases and controls (Table 6.6). Forty eight percent of cases were men compared with 47% of controls. There was no significant difference in mean age between cases (71.6 years) and controls (70.9 years) in the total study sample, nor between male cases and controls (71.6 vs 71.4 years) or female cases and controls (71.3 vs 70.5 years).

Increasing number of cases were recruited from successive age bands between the ages of 60 and 74 years. This was not surprising given the increase in prevalence of peripheral arterial disease with age. However, fewer subjects were recruited from the oldest (75 to 80 year) age band. This appeared to be a combination of the lower number of subjects in this age band attending the Edinburgh Artery Study 5-year follow-up examination plus a lower response rate in older groups (see Tables 6.2 and 6.3). As matching was done by age bands, the percentage of the sample from each of the four age bands was no different between cases and controls.

Mean levels of a number of risk factors were compared between cases and controls, both in the total study sample and after the sample was split by sex. This included the 'established' cardiovascular risk factors (cigarette smoking, hypertension and hyperlipidaemia) as well as blood glucose and measures of obesity (body mass index

and waist hip ratio). These latter factors were included because of their recognised associations with plasma insulin and oestrogen levels respectively. The results are summarised in Table 6.7 (both sexes combined), Table 6.8 (males) and Table 6.9 (females); the main findings are described below.

Mean lifetime smoking, measured in square root pack-years to normalise the skewed distribution of pack-years within the study population, was significantly higher in cases (3.08; 95% CI 2.38, 3.78) than in controls (1.81; 95% CI 1.27, 2.34, $p \leq 0.01$). When the population was split by sex, higher levels of smoking were found in both male and female cases compared with controls, although the differences were less significant.

Systolic and diastolic blood pressure measurements were found to be normally distributed within the study population and so did not require transformation prior to analysis. Mean systolic blood pressure was significantly higher in cases (154.8 mmHg; 95% CI 149.3, 160.3) than in controls (143.6 mmHg; 95% CI 138.7, 148.5; $p \leq 0.01$). Mean diastolic pressure was also higher in cases (83.6 mmHg; 95% CI 81.1, 86.2) compared with controls (80.6 mmHg; 95% CI 78.3, 82.9) but this difference was less marked and significant at the 10% level only ($p=0.07$). When the distribution of blood pressure was examined in the two sexes separately, the higher mean levels of systolic pressure in cases was confined almost exclusively to men (155.5 mmHg vs 138.7 mmHg; $p \leq 0.01$); there was no significant difference in mean systolic pressure between female cases and controls (154.2 mmHg vs 147.8 mmHg, $p > 0.1$).

Serum levels of total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides were found to be normally distributed except for serum tryglycerides which was positively skewed (necessitating log transformation prior to analysis). There was no significant difference between total cases and controls in mean levels of total cholesterol (6.52 mmol/l vs 6.57 mmol/l, $p>0.1$) or low density lipoprotein cholesterol (4.58 mmol/l vs 4.63 mmol/l, $p>0.1$). Similar levels of these variables were also found in male and female cases and controls when the sample was split by sex.

Mean high density lipoprotein cholesterol was slightly lower in cases (1.20 mmol/l; 95% CI 1.12, 1.27) than in controls (1.29 mmol/l; 95% CI 1.22, 1.36), although this difference was only significant at the 10% level ($p=0.07$). This was predominantly due to lower levels in female cases than controls (1.31 mmol/l vs 1.44 mmol/l, $p=0.06$); there was no significant difference between male cases and controls (1.07 mmol/l vs 1.12 mmol/l, $p>0.1$).

The greatest difference in serum lipid levels between cases and controls was found for serum triglycerides. In the total study sample, mean levels were considerably higher in cases (1.52 mmol/l; 95% CI 1.39, 1.67) than in controls (1.34 mmol/l; 95% CI 1.23, 1.45 $p\leq0.05$). This was predominantly due to higher levels in male cases than controls (1.53 mmol/l vs 1.28 mmol/l, $p=0.07$); there was no significant difference between female cases and controls (1.50 mmol/l vs 1.39 mmol/l, $p>0.1$).

Distributions of fasting blood glucose and one-hour blood glucose (blood glucose one hour after an oral glucose load) were positively skewed, necessitating log transformation prior to analysis. There was no significant difference in mean fasting glucose between cases and controls in the total study sample (5.5 mmol/l vs 5.6 mmol/l, $p>0.1$), nor when the sample was split by sex. Mean one-hour glucose was higher in total cases (8.9 mmol/l; 95% CI 8.4, 9.6) than in controls (8.1 mmol/l; 95% CI 7.6, 8.6, $p\leq0.05$), but there was no significant difference between cases and controls when mean levels were compared separately in men (9.0 mmol/l vs 8.3 mmol/l, $p>0.1$) and in women (8.8 mmol/l vs 7.9 mmol/l, $p>0.1$).

Body mass index was normally distributed and so did not require transformation prior to analysis. In the total study sample, there was no significant difference in mean body mass index between cases and controls (26.3 kg/m^2 vs 25.9 kg/m^2, $p>0.1$). However, when the sample was split by sex, male cases had a slightly higher mean body mass index than controls (26.5 kg/m^2 vs 25.2 kg/m^2, $p=0.06$). No such difference was found amongst female subjects.

Waist hip ratio (the ratio of waist circumference to hip circumference) was also normally distributed. In the total study sample, the difference in mean waist hip ratio between cases (0.90; 95% CI 0.88, 0.92) and controls (0.88; 95% CI 0.86,0.90) did not quite reach the 10% significance level ($p=0.12$). However, mean waist hip ratio *was* found to be significantly higher in male cases than controls (0.92 vs 0.89, $p\leq0.05$). Female cases and controls had a similar mean waist hip ratio (0.88 vs 0.87, $p>0.1$).

In summary: In the total study population, cases smoked more than controls and had higher mean systolic blood pressure, serum triglycerides and one-hour blood glucose. Cases also had slightly higher mean diastolic blood pressure and slightly lower mean high density lipoprotein cholesterol. When the total study population was split by sex, male cases had higher mean systolic blood pressure and waist hip ratio than male controls. They also had slightly higher lifetime smoking, mean serum triglycerides and mean body mass index. In the female study population, cases smoked more than controls and had slightly lower mean high density lipoprotein cholesterol.

6.2 Plasma insulin

6.2.1 Univariate analysis

Mean levels of fasting plasma insulin, plasma insulin one hour after the oral glucose load (one-hour insulin) and insulin resistance in cases and controls are given in Table 6.10. For all of these variables, the levels were log transformed because of skewed distributions (Figures 6.1 to 6.3). Geometric means and transformed confidence intervals have therefore been presented.

There was no significant difference in mean fasting insulin between cases and controls, either in the total study population (6.4 mU/l vs 6.1 mU/l, $p>0.1$) or when the sample was split by sex (men 6.9 mU/l vs 5.8 mU/l, $p>0.1$; women 5.9 mU/l vs 6.3 mU/l,

p>0.1). However, mean insulin, one hour after the oral glucose load, was significantly higher in total cases (73.6 mU/l; 95% CI 65.0, 83.3) than in controls (59.8 mU/l; 95%CI 53.2, 67.3, p≤0.05). When one-hour insulin levels were compared between cases and controls in men and women separately, male cases had slightly higher mean levels than male controls (81.7 mU/l vs 65.0 mU/l; p=0.06). However, the difference between female cases and controls did not quite reach the 10% significance level (66.6 mU/l vs 55.6 mU/l; p=0.11). Mean insulin resistance was not significantly different between total cases and controls (1.55 vs 1.49, p>0.1), nor between male or female cases and controls.

Correlations between one-hour plasma insulin and the other cardiovascular risk factors are given for cases and controls separately in Table 6.11. The most marked correlations, present in both the case and control groups, were found between one-hour insulin and smoking (r = 0.26, p≤0.05 in cases; r = 0.27, p≤0.05 in controls), serum high density lipoprotein cholesterol (r = -0.28, p≤0.01 in cases; r = -0.22, p≤0.05 in controls), serum triglycerides (r = 0.18, p≤0.1 in cases; r = 0.28, p≤0.01 in controls), fasting blood glucose (r = 0.21, p≤0.1 in cases; r = 0.36, p≤0.001 in controls) and one-hour blood glucose (r = 0.34, p≤0.01 in cases; r = 0.49, p≤0.001 in controls). In the control group, one-hour insulin also correlated with diastolic blood pressure (r = 0.39, p≤0.001), serum low density lipoprotein cholesterol (r = 0.24, p≤0.05) and weakly with total serum cholesterol (r = 0.19, p≤0.1), but these factors did not correlate with one-hour insulin in the cases.

6.2.2 Multivariate analysis

The results of multiple logistic regression, used to examine the association between one-hour plasma insulin concentration and risk of peripheral arterial disease, before and after adjustment for the other cardiovascular risk factors, are shown in Table 6.12. Higher one-hour insulin levels were associated with a significant increase in the risk of peripheral arterial disease (odds ratio of disease for a one log unit increase in one hour insulin concentration, adjusted for age and sex was 1.96; 95% CI, 1.11, 3.44; $p \leq 0.05$). The strength of the association between disease and one hour insulin was not significantly different between men (odds ratio 2.24; 95% CI, 1.00, 5.06) and women (odds ratio 1.82; 95% CI, 0.78, 4.30: p-value for difference between odds ratios >0.1). Because of this finding, and to prevent loss of power due to small numbers, further multivariate adjustment was performed only with the two sexes combined.

Adjustment for the effects of systolic and diastolic blood pressure (with the sexes combined) did not greatly alter the association between disease and one-hour insulin concentration. Thus the odds ratio changed only marginally from 1.96 (95% CI 1.11, 3.44) to 2.04 (95% CI 1.11, 3.74), the 95% confidence intervals of these two ratios overlapped almost entirely and the effect of insulin on disease remained significant (adjusted odds ratio 2.04, $p \leq 0.05$).

Further adjustment for the effects of plasma low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides slightly weakened the relationship between insulin and disease (odds ratio fell from 2.04; 95%CI 1.11, 3.74, to 1.86; 95%

CI 0.99, 3.48). However, the 95% confidence intervals of these two ratios overlapped considerably and the effect of insulin on disease remained significant at the 10% level (multi-adjusted odds ratio 1.86, $p \leq 0.1$).

The addition of smoking to the multivariate model had the greatest effect on the relationship between insulin and disease. Adjustment for the effects of blood pressure, serum lipids *and* smoking resulted in a reduction in the odds ratio to 1.64 (95% CI 0.83, 3.23). Although this was not a large drop from the previously adjusted ratio of 1.86 (and there was still considerable overlap in the 95% confidence intervals), the ratio became non-significant ($p=0.17$).

Despite the high level of correlation between one-hour glucose and insulin levels, addition of one-hour glucose to the multivariate models in Table 6.13 made very little difference to the odds ratios for one-hour insulin and disease. After adjustment for age, sex, blood pressure and one-hour blood glucose, higher one-hour insulin levels remained associated with a significant increase in the risk of peripheral arterial disease (odds ratio 1.96; 95% CI, 1.00, 3.82; $p \leq 0.05$). Following further adjustment for serum lipids, the odds ratio fell to 1.86 (95% CI 0.94, 3.66), but remained significant at the 10% level ($p=0.08$). Conversely, one-hour glucose was not associated with an increased risk of peripheral arterial disease after adjustment for age, sex and one-hour insulin (odds ratio 1.60; 95% CI, 0.52,4.93; $p>0.1$).

6.2.3 Insulin, smoking and disease

The weakening of the relationship between one-hour insulin and peripheral arterial disease when smoking was added to the multivariate model suggested that raised plasma insulin levels might be an intermediary factor in the influence of smoking on the development of disease. To explore this possibility further, mean one-hour insulin was compared between ever-smokers (current and ex-smokers) and never-smokers in both cases and controls (Figure 6.4). Mean one-hour insulin was significantly higher in ever-smokers than in never-smokers in both cases (81.9 mU/l; 95%CI 67.2, 99.9 vs 61.6 mU/l; 95%CI 53.8, 71.1, $p\leq=0.03$) and controls (70.0 mU/l; 95%CI 58.7, 83.5 vs 49.4 mU/l; 95%CI 41.6, 58.5, $p\leq0.01$). This finding was consistent with the significant correlation between pack-years and one-hour insulin in cases ($r = 0.26$, $p\leq0.05$) and controls ($r = 0.27$, $p\leq0.05$).

Multivariate linear regression analysis was used to examine the independence of the relationship between one-hour plasma insulin levels and smoking status, after controlling for the other cardiovascular risk factors. There was a significant association between smoking (ever- versus never-smoker) and one-hour insulin after adjustment for the possible confounding factors of age, sex and peripheral arterial disease (B coefficient 0.26, SE 0.09; $p\leq0.01$). The association between smoking and one-hour insulin did not alter greatly after additional adjustment for the other cardiovascular risk factors, including systolic and diastolic blood pressure, low density lipoprotein and high density lipoprotein cholesterol and triglycerides (B coefficient 0.22, SE 0.09; $p\leq0.05$).

To determine whether raised insulin levels in smokers could help to explain the effects of smoking on the development of peripheral arterial disease, multiple logistic regression was used to calculate the odds of disease for a one √pack-year increase in smoking and then to adjust this for one-hour insulin levels. Despite the association between smoking and insulin, the relationship between peripheral arterial disease and smoking (age and sex adjusted odds ratio of disease for a one √pack-year increase in smoking 1.19; 95% CI, 1.05, 1.35; $p \leq 0.01$) was only very slightly weakened after this adjustment (odds ratio 1.16; 95% CI, 1.02, 1.32; $p \leq 0.05$). Further adjustment for serum high density lipoprotein cholesterol and triglyceride levels, which were correlated with smoking habit in subjects with peripheral arterial disease (Spearman's rank correlation coefficients; high density lipoprotein cholesterol $r = -0.32$, $p \leq 0.05$, triglycerides $r = 0.24$, $p \leq 0.05$) also had little effect on the association between smoking and disease (odds ratio 1.15; 95% CI 1.01, 1.31; $p \leq 0.05$).

In summary: Compared with controls, cases had higher mean plasma insulin levels one hour after the oral glucose load (73.6 mU/l vs 59.8 mU/l; $p \leq 0.05$), but not fasting levels (6.4 mU/l vs 6.1 mU/l; $p > 0.1$). The relationship between one-hour plasma insulin and disease was independent of blood pressure (odds ratio 2.04; 95% CI 1.11,3.74; $p \leq 0.05$) and partially independent of serum low and high density lipoprotein cholesterol and triglycerides (odds ratio 1.86; 95% CI 0.99, 3.48; $p \leq 0.1$). However, when smoking was added to the multivariate model, the relationship between insulin and disease diminished (odds ratio 1.64; 95% CI 0.84,3.23; $p > 0.1$), consistent with raised mean insulin levels in smokers.

6.3 Steroid sex hormones

6.3.1 Univariate analysis

Mean levels of steroid sex hormones were compared between cases and controls. For plasma sex hormone-binding globulin and plasma oestrone, levels were log transformed in both men and women because of skewed distributions. Geometric means and transformed confidence intervals were given for these variables. All of the other variables were normally distributed in both sexes. All analyses were performed in men and women separately because of their unique sex hormone profiles.

Mean levels of steroid sex hormones in male cases and controls are given in Table 6.13. There was no significant difference between cases and controls in mean levels of total plasma testosterone (13.6 nmol/l vs 14.9 nmol/l, p=0.3), plasma sex hormone-binding globulin (34.6 nmol/l vs 39.5 nmol/l, p=0.12) or free plasma testosterone (403.4 pmol/l vs 379.2 pmol/l, p=0.5). Neither was there any significant difference in mean plasma oestradiol (97.9 pmol/l in cases compared with 106.1 pmol/l in controls, p=0.2). Mean levels of plasma oestrone were slightly higher in cases (101.9 pmol/l; 95% CI 94.8, 109.5) than controls (92.1 pmol/l, 95% CI 84.2, 100.8) but this difference was only significant at the 10% level (p=0.09).

Mean levels of steroid sex hormones in female cases and controls are given in Table 6.14. There was no significant difference between cases and controls in mean levels of total plasma testosterone (0.62 nmol/l vs 0.72 nmol/l, p=0.3), plasma sex hormone-

binding globulin (45.0 nmol/l vs 49.0 nmol/l, p=0.5), or free plasma testosterone (15.3 pmol/l vs 16.0 pmol/l, p=0.8). Neither was there any significant difference in mean plasma oestrone (81.26 pmol/l in cases compared with 82.96 pmol/l in controls, p=0.7) or plasma oestradiol (47.02 pmol/l in cases compared with 49.72 pmol/l in controls, p=0.4).

The prevalence of thyroid disease, which can affect sex hormone-binding globulin concentrations, was low. Five female cases, two female controls and no male subjects were taking thyroxine (which can increase levels of the globulin) and no subjects of either sex were being treated for hypothyroidism. When female subjects taking thyroxine were excluded from the analysis, there remained no significant difference in mean plasma sex hormone-binding globulin between cases and controls (44.6 nmol/l vs 48.6 nmol/l, p=0.5).

Spearman's rank correlation coefficients were calculated to examine associations between steroid sex hormones and the other cardiovascular risk factors in male cases and controls (Tables 6.15 and 6.16) and in female cases and controls (Tables 6.17 and 6.18). In male cases, plasma sex hormone-binding globulin correlated significantly with serum high density lipoprotein cholesterol ($r = 0.39$, $p \leq 0.05$) and negatively with serum triglycerides ($r = -0.34$, $p \leq 0.05$), body mass index ($r = -0.40$, $p \leq 0.05$) and waist hip ratio ($r = -0.48$, $p \leq 0.01$), but these correlations were not evident in male controls. Total plasma testosterone also correlated with body mass index ($r = -0.39$, $p \leq 0.05$) and waist hip ratio ($r = -0.31$, $p \leq 0.05$) and free plasma testosterone with waist hip ratio ($r = 0.33$, $p \leq 0.05$) in male cases but not in controls. The only other significant association in

men was a negative correlation between free plasma testosterone and diastolic blood pressure in cases ($r = -0.36$, $p \leq 0.05$).

In women, sex hormone-binding globulin correlated in controls with diastolic blood pressure ($r = -0.44$, $p \leq 0.01$), in cases with serum high density lipoprotein cholesterol ($r = 0.38$, $p \leq 0.01$) and in both groups with serum triglycerides ($r = -0.35$, $p \leq 0.05$ in cases, $r = -0.47$, $p \leq 0.001$ in controls) and body mass index ($r = -0.44$, $p \leq 0.01$ in cases, $r = -0.46$, $p \leq 0.001$ in controls). Free plasma testosterone correlated in cases with lifetime smoking ($r = 0.38$, $p \leq 0.05$), in both groups with diastolic blood pressure ($r = 0.31$, $p \leq 0.05$ in cases, $r = 0.29$, $p \leq 0.05$ in controls) and in controls with total serum cholesterol ($r = 0.45$, $p \leq 0.01$), serum low density lipoprotein cholesterol ($r = 0.42$, $p \leq 0.01$) and serum triglycerides ($r = 0.45$, $p \leq 0.01$). Total plasma testosterone correlated in cases with lifetime smoking ($r = 0.42$, $p \leq 0.01$) and in controls with total serum cholesterol ($r = 0.38$, $p \leq 0.01$) and serum low density lipoprotein cholesterol ($r = 0.36$, $p \leq 0.05$).

6.3.2 Multivariate analysis

Multiple logistic regression was used to examine the association between steroid sex hormones and risk of disease after adjustment for the other cardiovascular risk factors. The odds of disease was adjusted first for body mass index because of the close relationship between obesity and sex hormones, in particular plasma oestrogens. Adjustment was then made for the other selected risk factors linked with an increased

risk of cardiovascular disease (systolic blood pressure, smoking, serum low density and high density lipoprotein cholesterol and serum triglycerides).

In men, none of the sex hormones was associated with an increased or decreased risk of peripheral arterial disease after multivariate adjustment for age and body mass index (Table 6.19). This included plasma oestrone, for which the unadjusted mean levels had been marginally higher in cases than in controls (odds ratio associated with a one log unit increase in oestrone 3.36; 95% CI 0.55, 20.6, p=0.2). For the other hormones, odds ratios ranged from 0.43 (95% CI 0.12, 1.55) for plasma sex hormone-binding globulin to 1.21 (95% CI 0.74, 1.99) for free plasma testosterone. Further adjustment for systolic blood pressure, smoking, serum low density cholesterol, serum high density lipoprotein cholesterol and serum triglycerides did not alter the odds ratios greatly (range from 0.50; 95% CI 0.10, 2.60 for plasma sex hormone-binding globulin to 1.10; 95% CI 0.61, 1.99 for free plasma testosterone). Replacement of body mass index with waist hip ratio (as a measure of regional obesity) in the multivariate model had no substantial impact on these results.

In women, findings were similar. None of the sex hormones was associated with risk of peripheral arterial disease after multivariate adjustment for age and body mass index (Table 6.20). Odds ratios ranged from 0.59 (95% CI 0.23, 1.49) for plasma sex hormone-binding globulin to 1.98 (95% CI 0.63, 2.51) for free plasma testosterone. Further adjustment for systolic blood pressure, smoking, serum low density lipoprotein cholesterol, serum high density lipoprotein cholesterol and serum triglycerides did not alter the odds ratios greatly (range from 0.70; 95% CI 0.41, 1.17 for total plasma

testosterone to 0.79; 95% CI 0.47, 1.32 for plasma oestradiol). Again, replacement of body mass index with waist hip ratio in the multivariate model had no substantial impact on the results.

In summary: Mean plasma oestrone levels were slightly higher in male cases than controls (101.9 pmol/l vs 92.1 pmol/l; p=0.09), but this association lost significance after multivariate adjustment for age and body mass index. Mean levels of plasma total and free testosterone, oestradiol, and sex hormone-binding globulin were not significantly different in cases compared with controls in either sex (p>0.1).

Table 6.1. Age, sex and social class distribution in subjects who attended the Edinburgh Artery Study 5-year follow-up examination and in the total study population eligible to attend

	Percentage (or mean), n	
	Attenders (n=1156)	***Total eligible study population (n=1389)***
Sex		
Males	49.5 (572)	48.4 (672)
Age		
Mean years (SE)	69.7 (0.2)	69.8 (0.2)
60-64 years	25.4 (294)	24.9 (346)
65-69 years	27.6 (319)	26.8 (372)
70-74 years	25.8 (298)	26.4 (367)
75-79 years	21.2 (245)	21.9 (304)
Social class		
I	11.9 (138)	10.9 (151)
II	34.3 (396)	32.3 (449)
IIIN	25.9 (299)	28.1 (390)
IIIM	17.4 (201)	16.3 (226)
IV	7.4 (86)	8.8 (122)
V	2.8 (32)	3.1 (43)

All differences between attenders and total eligible sample non-significant ($p>0.1$)
SE, standard error

Table 6.2. Percentage of subjects attending the Edinburgh Artery Study follow-up examination who were initially identified as eligible for participation in the case control study, by 5-year age band and sex

	Eligible subjects†, % (n)					
	Total		***Men***		***Women***	
	Cases	***Controls***	***Cases***	***Controls***	***Cases***	***Controls***
Age band						
60-64 years (n=294)	5.1 (15)	32.0 (94)	6.3 (8)	33.1 (42)	4.2 (7)	31.7 (53)
65-69 years (n=319)	7.2 (23)	28.8 (92)	4.2 (7)	31.9 (53)	10.5 (16)	25.5 (39)
70-74 years (n=298)	14.8 (44)	19.1 (57)	14.8 (23)	19.4 (30)	16.8 (24)	21.0 (30)
75-80 years (n=245)	17.1 (42)	17.6 (43)	12.9 (16)	19.4 (24)	22.3 (27)	15.7 (19)
All ages (n=1156)	11.1 (128)	25.1 (290)	9.4 (54)	26.0 (149)	12.7 (74)	24.1 (141)

† Includes subjects identified as eligible on the Edinburgh Artery Study database and subsequently found to be ineligible when they attended for examination (n= 4 cases, 4 controls)

Table 6.3. Response rates in cases and controls by 5-year age band and sex

	Response rates†, % (no. responders/ no. invited)					
	Total		***Men***		***Women***	
	Cases	***Controls***	***Cases***	***Controls***	***Cases***	***Controls***
Age band						
60-64 years	86.7 (13/15)	92.9 (13/14)	87.5 (7/8)	87.5 (7/8)	85.7 (6/7)	100 (6/6)
65-69 years	73.9 (17/23)	90.5 (19/21)	71.4 (5/7)	100 (5/5)	75.0 (12/16)	87.5 (14/16)
70-74 years	75.0 (30/44)	77.5 (31/40)	81.0 (17/21)	73.9 (17/23)	56.5 (13/23)	82.4 (14/17)
75-80 years	65.7 (23/35)	69.4 (25/36)	73.3 (11/15)	70.6 (12/17)	60.0 (12/20)	68.4 (13/19)
All ages	70.9 (83/117)	79.3 (88/111)	78.4 (40/51)	77.5 (41/53)	65.2 (43/66)	81.0 (47/58)

† Excludes those subjects invited for examination and subsequently found to be ineligible (n= 4 cases, 4 controls)

Table 6.4. Age, sex, social class distribution and peripheral arterial disease in case responders and total eligible case population

	Percentage (or mean), n	
	Case responders (n=83)	***Total eligible case population (n=124)***
Sex		
Males	48.2 (40)	41.1 (51)
Age		
Mean years (SE)	71.6 (0.6)	72.4 (0.5)
60-64 years	15.7 (13)	12.1 (15)
65-69 years	20.5 (17)	18.5 (23)
70-74 years	36.1 (30)	35.5 (44)
75-79 years	27.8 (23)	33.9 (42)
Social class		
I	9.6 (8)	8.1 (10)
II	45.8 (38)	40.3 (50)
IIIN	22.9 (19)	23.4 (29)
IIIM	13.3 (11)	17.7 (22)
IV	3.6 (3)	4.8 (6)
V	4.8 (4)	5.6 (7)
Intermittent claudication		
Grade 1	8.4 (7)	8.1 (10)
Grade 2	10.8 (9)	10.5 (13)
Probable	12.0 (10)	12.9 (16)
Total	31.3 (26)	31.5 (39)
ABPI		
Mean (SE)	0.71 (0.02)	0.72 (0.01)

All differences between responders and total eligible population non-significant ($p>0.1$)
ABPI, ankle brachial pressure index; SE, standard error

Table 6.5. Disease characteristics of cases of peripheral arterial disease and controls

	Percentage (or mean), n	
	Cases	*Controls*
Total		
Intermittent claudication		
Grade 1	8.4 (7)	0
Grade 2	10.8 (9)	0
Probable	12.0 (10)	0
Total	31.3 (26)	0
ABPI, mean (SE)	0.71 (0.02)	1.12 (0.01)
Coronary artery disease		
Angina	24.1 (20)	0
Myocardial infarction	16.9 (14)	0
Stroke	3.6 (3)	0
Men		
Peripheral arterial disease		
Intermittent claudication	30.0 (12)	0
ABPI, mean (SE)	0.70 (0.02)	1.14 (0.02)
Coronary artery disease		
Angina	27.5 (11)	0
Myocardial infarction	20.0 (8)	0
Women		
Peripheral arterial disease		
Intermittent claudication	32.6 (14)	0
ABPI, mean (SE)	0.72 (0.02)	1.10 (0.02)
Coronary artery disease		
Angina	20.9 (9)	0
Myocardial infarction	14.0 (6)	0

ABPI ankle brachial pressure index; SE, standard error
All differences in mean ABPI between cases and controls, $p \leq 0.001$

Table 6.6. Age and sex characteristics of cases of peripheral arterial disease and controls

	Percentage (or mean), n					
	Total		*Males*		*Females*	
	Cases (n=83)	*Controls (n=88)*	*Cases (n=40)*	*Controls (n=41)*	*Cases (n=43)*	*Controls (n=47)*
Sex						
Males	48.2 (40)	46.6 (41)	-	-	-	-
Age						
Mean years (SE)	71.6 (0.6)	70.9 (0.5)	71.9 (0.8)	71.4 (0.82)	71.3 (0.8)	70.5 (0.7)
60-64 years	15.7 (13)	14.8 (13)	17.5 (7)	17.1 (7)	14.0 (6)	12.8 (6)
65-69 years	21.7 (18)	21.6 (19)	12.5 (5)	12.2 (5)	30.2 (13)	29.8 (14)
70-74 years	36.1 (30)	35.2 (31)	42.5 (17)	41.5 (17)	27.9 (12)	29.8 (14)
75-80 years	28.9 (24)	28.4 (25)	27.5 (11)	29.3 (12)	27.9 (12)	27.7 (13)

All differences between cases and controls non-significant ($p > 0.1$)
SE, standard error

Table 6.7. Mean cardiovascular risk factors in cases of peripheral arterial disease and controls

Risk factor	*Mean (95% confidence interval)*		*p-value*
	Cases (n=83)	*Controls (n=88)*	
Smoking			
√packyears	3.08 (2.38, 3.78)	1.81 (1.27, 2.34)	≤0.01
Blood pressure			
Systolic blood pressure (mmHg)	154.8 (149.3, 160.3)	143.6 (138.7, 148.5)	≤0.01
Diastolic blood pressure (mmHg)	83.6 (81.1, 86.2)	80.6 (78.3, 82.9)	≤0.1
Serum lipids			
Total cholesterol (mmol/l)	6.52 (6.28, 6.76)	6.57 (6.33, 6.81)	NS
LDL cholesterol (mmol/l)	4.58 (4.36, 4.80)	4.63 (4.42, 4.84)	NS
HDL cholesterol (mmol/l)	1.20 (1.12, 1.27)	1.29 (1.22, 1.36)	≤0.1
Triglycerides (mmol/l)†	1.52 (1.39, 1.67)	1.34 (1.23, 1.45)	≤0.05
Blood glucose			
Fasting glucose (mmol/l)†	5.5 (5.4, 5.7)	5.6 (5.5, 5.7)	NS
One-hour glucose (mmol/l)†	8.9 (8.4, 9.6)	8.1 (7.6, 8.6)	≤0.05
Obesity			
Body mass index (kg/m^2)	26.3 (25.5, 27.1)	25.9 (25.2, 26.6)	NS
Waist hip ratio	0.90 (0.88, 0.92)	0.88 (0.86, 0.90)	NS

† geometric mean of logged variable and transformed confidence interval
NS not significant, p>0.1; LDL, low density lipoprotein; HDL, high density lipoprotein

Table 6.8. Mean cardiovascular risk factors in male cases of peripheral arterial disease and controls

Risk factor	*Mean (95% confidence interval)*				*p-value*
	Cases (n=40)		*Controls (n=41)*		
Smoking					
√packyears	4.12	(3.13,5.11)	2.85	(1.91,3.79)	≤0.1
Blood pressure					
Systolic blood pressure (mmHg)	155.5	(146.5,164.4)	138.7	(132.0,145.4)	≤0.05
Diastolic blood pressure (mmHg)	83.9	(79.4,87.9)	78.2	(73.9,82.5)	NS
Serum lipids					
Total cholesterol (mmol/l)	6.23	(5.91,6.55)	6.21	(5.88,6.54)	NS
LDL cholesterol (mmol/l)	4.38	(4.07,4.68)	4.48	(4.17,4.79)	NS
HDL cholesterol (mmol/l)	1.07	(0.97,1.17)	1.12	(1.03,1.21)	NS
Triglycerides (mmol/l)†	1.53	(1.32,1.78)	1.28	(1.13,1.45)	≤0.1
Blood glucose					
Fasting glucose (mmol/l)†*	5.65	(5.48,5.83)	5.58	(5.45,5.71)	NS
One-hour glucose (mmol/l)†	8.96	(8.12,9.88)	8.27	(7.58,9.01)	NS
Obesity					
Body mass index (kg/m^2)	26.5	(25.4,27.6)	25.2	(24.3,26.0)	≤0.1
Waist hip ratio	0.92	(0.90,0.94)	0.89	(0.87,0.91)	≤0.05

† geometric mean of logged variable and transformed confidence interval
NS not significant, p>0.1; LDL, low density lipoprotein; HDL, high density lipoprotein

Table 6.9. Mean cardiovascular risk factors in female cases of peripheral arterial disease and controls

Risk factor	*Mean (95% confidence interval)*				*p-value*
	Cases (n=43)		*Controls (n=47)*		
Smoking					
√packyears	2.14	(1.24,3.04)	1.03	(0.51,1.55)	≤0.05
Blood pressure					
Systolic blood pressure (mmHg)	154.2	(147.4,161.0)	147.8	(141.0,154.7)	NS
Diastolic blood pressure (mmHg)	83.3	(81.9,84.7)	82.7	(81.7,83.1)	NS
Lipids					
Total cholesterol (mmol/l)	6.81	(6.46,7.16)	6.88	(6.57,7.19)	NS
LDL cholesterol (mmol/l)	4.77	(4.45,5.08)	4.77	(4.49,5.05)	NS
HDL cholesterol (mmol/l)	1.31	(1.22,1.40)	1.44	(1.35,1.53)	≤0.1
Triglycerides (mmol/l)†	1.50	(1.33,1.71)	1.39	(1.26,1.43)	NS
Blood glucose					
Fasting glucose (mmol/l)†	5.36	(5.19,5.54)	5.57	(5.38,5.73)	NS
One hour glucose (mmol/l)†	8.84	(8.08,9.68)	7.93	(7.16,8.78)	NS
Obesity					
Body mass index (kg/m^2)	26.1	(24.9,27.4)	26.5	(25.4,27.6)	NS
Waist hip ratio	0.88	(0.85,0.90)	0.87	(0.85,0.90)	NS

† geometric mean of logged variable and transformed confidence interval
NS not significant, p>0.1; LDL, low density lipoprotein; HDL, high density lipoprotein

Table 6.10. Mean levels of plasma insulin and insulin resistance in cases of peripheral arterial disease and controls (by sex)

	Mean, 95% confidence interval[†]				
	Cases		*Controls*		*p-value*
Total					
Fasting insulin (mU/l)	6.4	(5.8, 7.1)	6.1	(5.5, 6.7)	NS
One-hour insulin (mU/l)	73.6	(65.0, 83.3)	59.8	(53.2, 67.3)	≤0.05
Insulin resistance	1.55	(1.38, 1.75)	1.49	(1.34, 1.66)	NS
Men					
Fasting insulin (mU/l)	6.85	(5.77,8.14)	5.76	(5.01,6.62)	NS
One-hour insulin (mU/l)	81.74	(66.79,100.02)	65.03	(55.05,76.82)	≤0.1
Insulin resistance	1.72	(1.43,2.07)	1.43	(1.24,1.65)	NS
Women					
Fasting insulin (mU/l)	5.94	(5.19,6.80)	6.27	(5.49,7.16)	NS
One-hour insulin (mU/l)	66.57	(57.92,76.51)	55.57	(47.23,65.39)	NS
Insulin resistance	1.41	(1.21,1.66)	1.55	(1.33,1.81)	NS

[†] geometric mean of logged variable and transformed confidence interval

Figure 6.1 Frequency distribution of fasting insulin

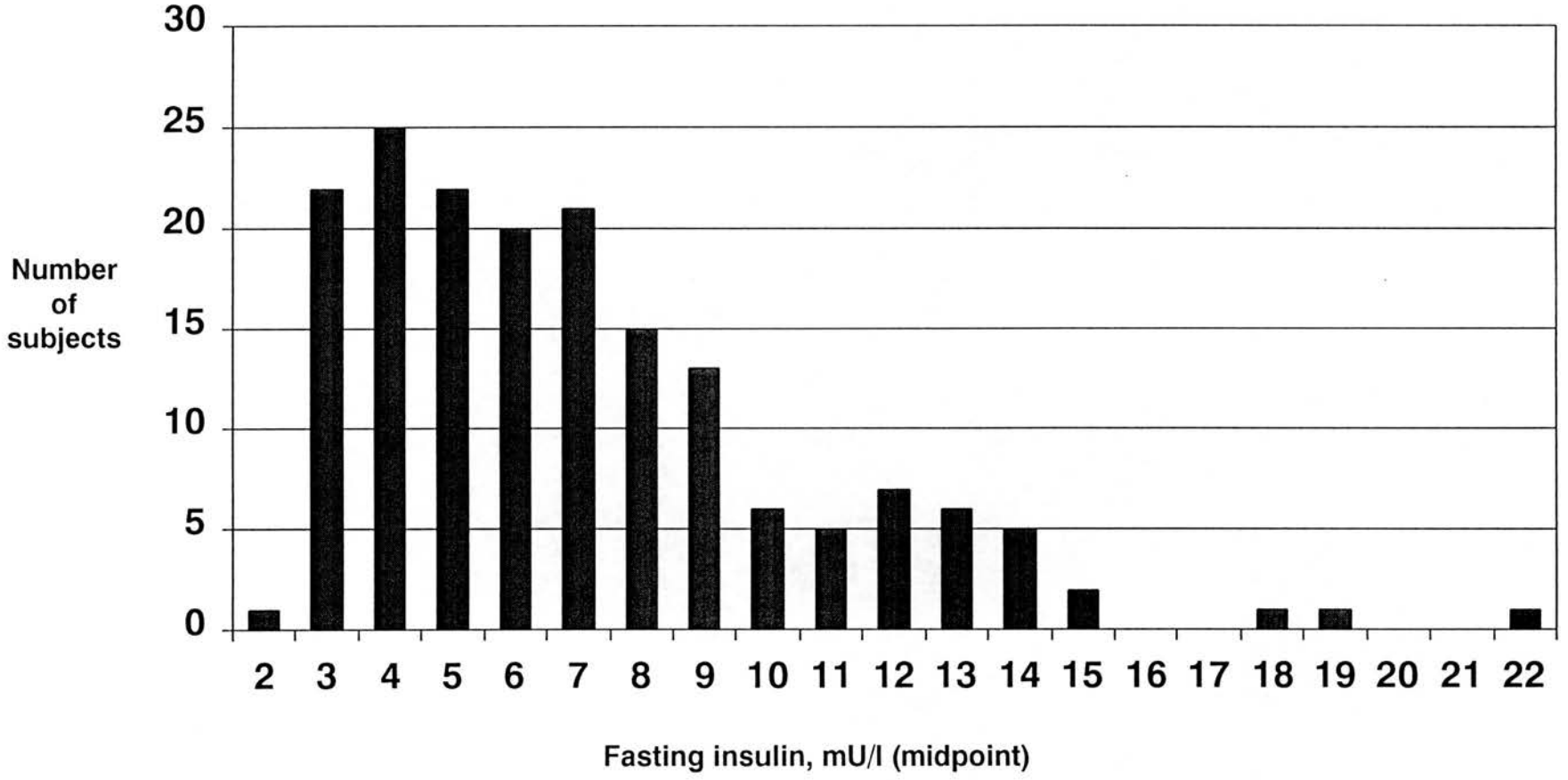


Figure 6.2 Frequency distribution of one-hour insulin

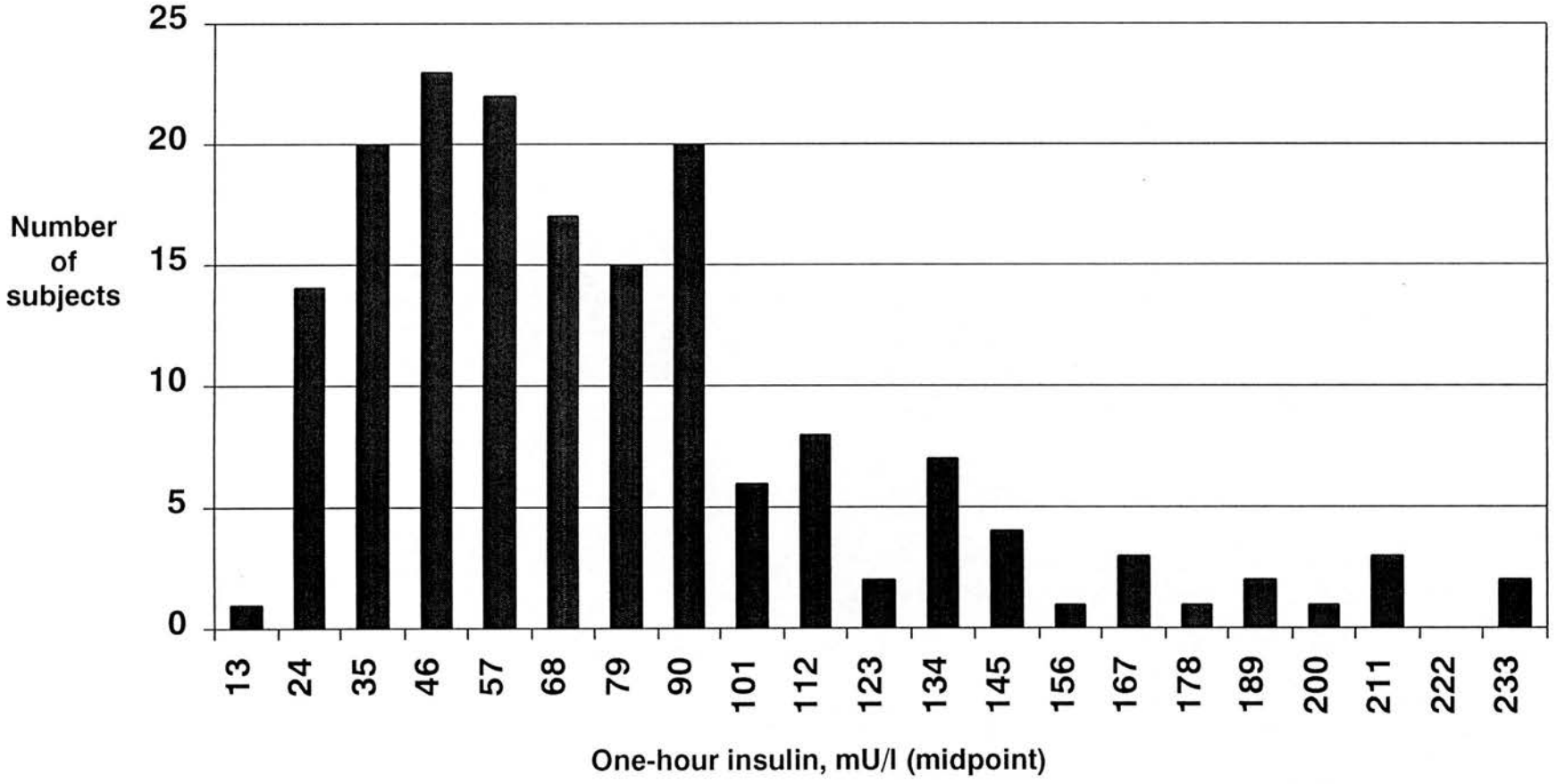


Figure 6.3 Frequency distribution of insulin resistance

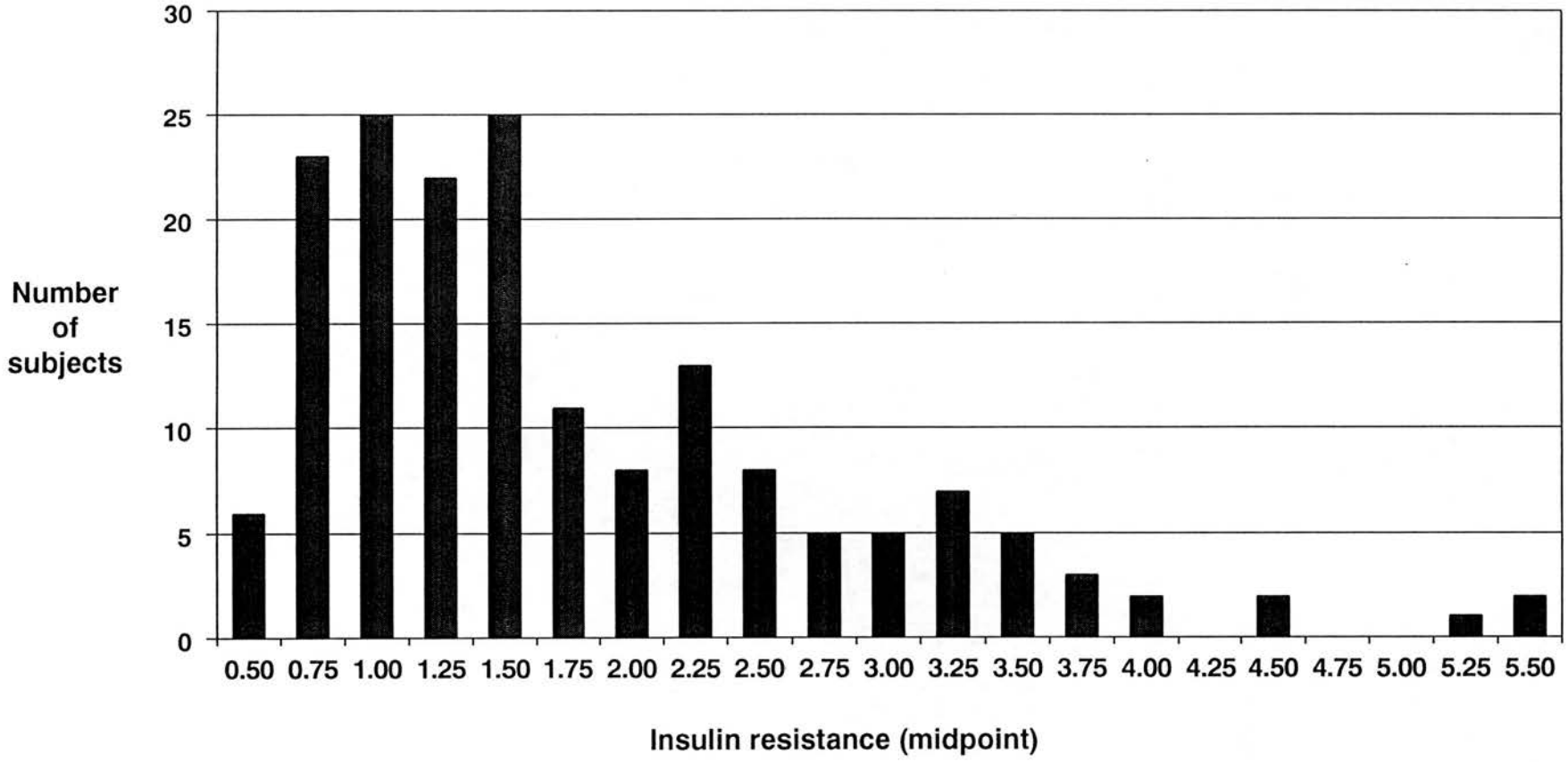


Table 6.11. Spearman's rank correlation coefficients of one-hour plasma insulin levels with cardiovascular risk factors in cases and controls

Risk factor	*One-hour insulin, mU/l*	
	Cases	*Controls*
Smoking		
Packyears	0.26**	0.27**
Blood pressure		
Systolic blood pressure, mmHg	-0.003	0.08
Diastolic blood pressure, mmHg	0.16	0.39****
Serum lipids		
Total cholesterol, mmol/l	-0.06	0.19*
LDL cholesterol, mmol/l	-0.06	0.24**
HDL cholesterol, mmol/l	-0.28***	-0.22**
Triglycerides, mmol/l	0.18*	0.28***
Blood glucose		
Fasting glucose, mmol/l	0.21*	0.36****
One-hour glucose, mmol/l	0.34**	0.49***
Obesity		
Body mass index, kg/m^2	0.30***	0.39****
Waist hip ratio	0.17	0.41****

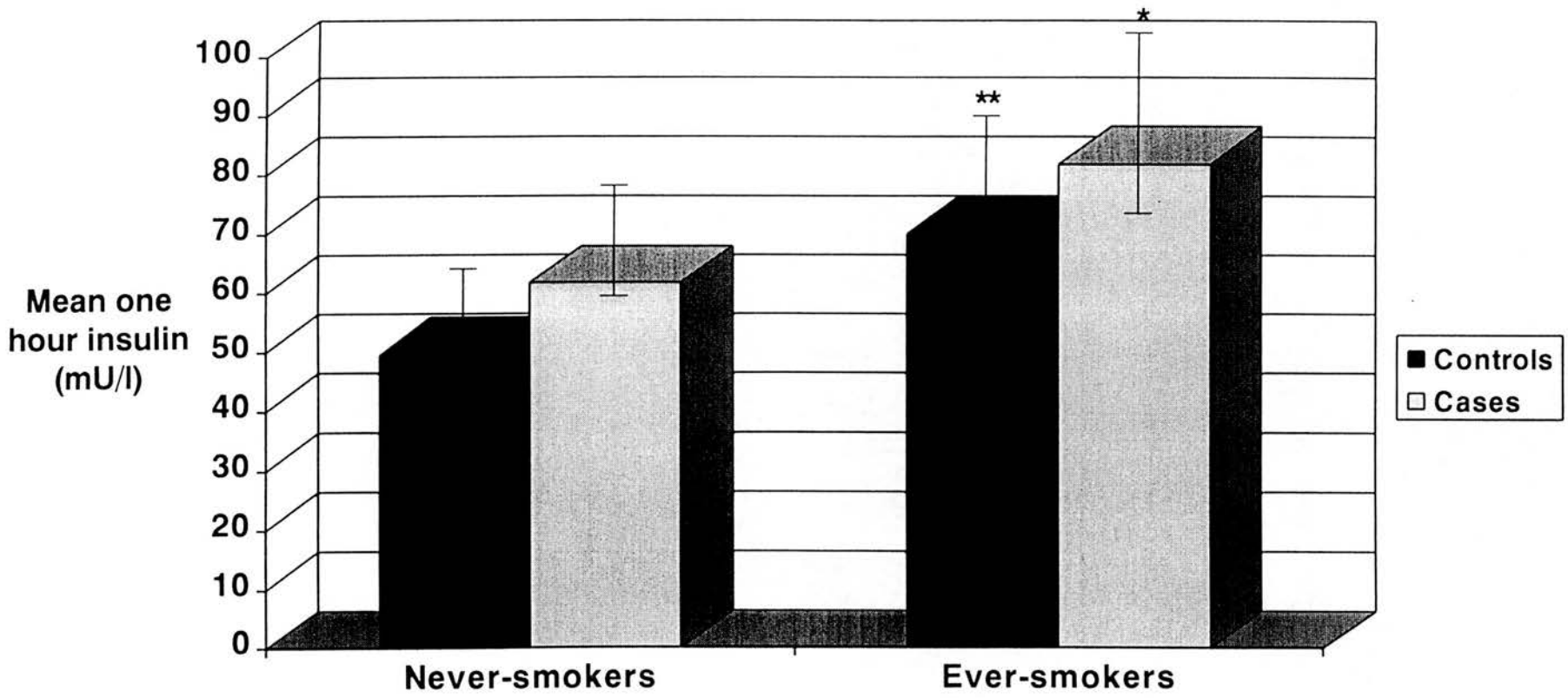
*$p \leq 0.1$, **$p \leq 0.05$, ***$p \leq 0.01$, ****$p \leq 0.001$

Table 6.12. Multivariate logistic regression of one-hour plasma insulin levels on peripheral arterial disease (all odds ratios adjusted for age and sex)

Risk factors in model[†]	*Odds ratio*[‡]	*95%CI*	*p-value*
-	1.96	(1.11,3.44)	≤0.05
Blood pressure	2.04	(1.11,3.74)	≤0.05
Blood pressure, LDL and HDL cholesterol, triglycerides	1.86	(0.99,3.48)	≤0.1
Blood pressure, LDL and HDL cholesterol, triglycerides, smoking	1.64	(0.83,3.23)	0.17

[†] in addition to age and sex
[‡] odds ratio for a one unit increase on a log scale in one-hour insulin
Variables included in model: systolic and diastolic blood pressure (blood pressure), serum low density lipoprotein and high density lipoprotein cholesterol (LDL and HDL cholesterol) and pack-years as a measure of lifetime smoking (smoking)
CI, confidence interval

Figure 6.4 Geometric mean (transformed 95% confidence intervals) of one-hour insulin levels in never-smokers and ever-smokers, according to disease status



*p≤0.05, **p≤0.01 (ever-smoker versus never-smoker)

Table 6.13. Mean levels of steroid sex hormones in male cases and controls

	Mean (95% confidence interval)				
	Cases (n=40)		*Controls (n=41)*		*p-value*
Plasma testosterone					
Total, nmol/l	13.6	(12.0,15.3)	14.9	(13.3,16.5)	NS
Free testosterone, pmol/l	403.4	(354.6,452.2)	379.2	(336.4,422.0)	NS
Plasma SHBG[†], nmol/l	34.6	(30.4,39.3)	39.5	(35.6,43.8)	0.12
Plasma oestrogens					
Oestrone[†], pmol/l	101.9	(94.8,109.5)	92.1	(84.2,100.8)	0.09
Oestradiol, pmol/l	97.9	(88.6,107.3)	106.1	(97.3,114.9)	NS

[†]geometric mean of logged variable
SHBG, sex hormone-binding globulin; NS, not significant ($p > 0.1$)

Table 6.14. Mean levels of steroid sex hormones in female cases and controls

	Mean (95% confidence interval)		
	Cases (n=43)	*Controls (n=47)*	*p-value*
Plasma testosterone			
Total, nmol/l	0.62 (0.50,0.73)	0.72 (0.58,0.86)	NS
Free, pmol/l	15.3 (11.24,19.35)	16.0 (12.4,19.5)	NS
Plasma SHBG[†], nmol/l	45.0 (38.2,53.1)	49.0 (42.4,56.7)	NS
Plasma oestrogens			
Oestrone[†], pmol/l	81.26 (73.96,89.27)	82.96 (77.92,88.33)	NS
Oestradiol, pmol/l	47.02 (42.76,51.29)	49.72 (45.44,54.00)	NS

[†]geometric mean of logged variable
SHBG, sex hormone-binding globulin; NS, not significant ($p > 0.1$)

Table 6.15. Spearman's rank correlation coefficients of plasma testosterone and sex hormone-binding globulin with cardiovascular risk factors in male cases and controls

	Total testosterone, nmol/l		*Free testosterone, pmol/l*		*SHBG, nmol/l*	
Risk factor	***Cases***	***Controls***	***Cases***	***Controls***	***Cases***	***Controls***
Smoking						
Packyears	0.12	-0.15	0.15	-0.07	-0.12	-0.15
Blood pressure						
Systolic blood pressure, mmHg	-0.12	0.22	-0.14	-0.19	0.08	0.29
Diastolic blood pressure, mmHg	-0.15	0.20	-0.36*	-0.13	0.26	0.24
Serum lipids						
Total cholesterol, mmol/l	-0.14	0.06	-0.15	-0.05	0.06	0.09
LDL cholesterol, mmol/l	-0.11	0.06	-0.16	-0.08	0.11	0.10
HDL cholesterol, mmol/l	0.26	0.24	-0.29	0.16	0.39*	-0.002
Triglycerides, mmol/l	-0.23	-0.12	0.13	-0.07	-0.34*	0.001
Obesity						
Body mass index, kg/m^2	-0.39*	0.07	0.18	-0.14	-0.40*	0.12
Waist hip ratio	-0.31*	0.04	0.33*	-0.08	-0.48**	0.05

* $p \leq 0.05$, ** $p \leq 0.01$; SHBG, sex hormone-binding globulin, LDL, low density lipoprotein; HDL, high density lipoprotein

Table 6.16. Spearman's rank correlation coefficients of plasma oestrogens with cardiovascular risk factors in male cases and controls

Risk factor	*Oestrone, pmol/l*		*Oestradiol, pmol/l*	
	Cases	*Controls*	*Cases*	*Controls*
Smoking				
Packyears	0.08	-0.04	0.07	-0.16
Blood pressure				
Systolic, mmHg	0.11	-0.12	0.12	0.25
Diastolic, mmHg	-0.41	-0.37	0.02	0.09
Serum lipids				
Total cholesterol, mmol/l	0.04	-0.34	-0.17	-0.11
LDL cholesterol, mmol/l	-0.07	-0.33	-0.20	-0.06
HDL cholesterol, mmol/l	0.01	0.15	0.21	0.26
Triglycerides, mmol/l	0.21	-0.16	-0.18	-0.26
Obesity				
Body mass index, kg/m^2	0.25	0.09	-0.11	0.16
Waist hip ratio	-0.01	-0.24	0.02	0.08

* $p \leq 0.05$, ** $p \leq 0.01$; LDL, low density lipoprotein; HDL, high density lipoprotein

Table 6.17. Spearman's rank correlation coefficients of plasma testosterone and sex hormone-binding globulin with cardiovascular risk factors in female cases and controls

	Total testosterone, nmol/l		*Free testosterone, pmol/l*		*SHBG, nmol/l*	
Risk factor	***Cases***	***Controls***	***Cases***	***Controls***	***Cases***	***Controls***
Smoking						
Packyears	0.42**	-0.01	0.38*	0.09	-0.16	-0.15
Blood pressure						
Systolic blood pressure, mmHg	0.12	0.15	0.26	0.19	-0.24	-0.07
Diastolic blood pressure, mmHg	0.15	0.06	0.31*	0.29*	-0.22	-0.44**
Lipids						
Total cholesterol, mmol/l	0.15	0.38**	0.15	0.45**	-0.05	-0.21
LDL cholesterol, mmol/l	0.12	0.36*	0.12	0.42**	-0.06	-0.16
HDL cholesterol, mmol/l	0.23	0.02	-0.09	-0.10	0.38**	0.22
Triglycerides, mmol/l	0.01	0.25	0.23	0.45**	-0.35*	-0.47***
Obesity						
Body mass index, kg/m^2	-0.15	-0.11	0.18	0.13	-0.44**	-0.46***
Waist hip ratio	0.08	-0.04	0.10	-0.01	-0.12	-0.07

* $p \leq 0.05$, ** $p \leq 0.01$; SHBG, sex hormone-binding globulin, LDL, low density lipoprotein; HDL, high density lipoprotein

Table 6.18. Spearman's rank correlation coefficients of plasma oestrogens with cardiovascular risk factors in female cases and controls

Risk factor	*Oestradiol, pmol/l*		*Oestrone, pmol/l*[†]	
	Cases	*Controls*	*Cases*	*Controls*
Smoking				
Packyears	-0.05	0.13	0.12	-0.11
Blood pressure				
Systolic blood pressure, mmHg	0.06	0.15	0.17	0.27
Diastolic blood pressure, mmHg	0.11	0.02	0.13	0.26
Lipids				
Total cholesterol, mmol/l	-0.16	-0.0005	-0.19	0.10
LDL cholesterol, mmol/l	-0.12	-0.07	-0.13	0.03
HDL cholesterol, mmol/l	0.17	-0.01	0.20	-0.01
Triglycerides, mmol/l	-0.26	0.16	-0.27	0.19
Obesity				
Body mass index, kg/m^2	0.04	-0.05	-0.07	0.18
Waist hip ratio	0.07	-0.12	-0.12	-0.09

[*] $p \leq 0.05$, [**] $p \leq 0.01$; LDL, low density lipoprotein; HDL, high density lipoprotein

Table 6.19. Multivariate logistic regression of steroid sex hormones on peripheral arterial disease in men

Hormone (SD or log unit) factors adjusted for	*Odds ratio (95% CI)*[†]	*p-value*
Total testosterone (5.22 nmol/l)		
age, BMI	0.84 (0.52,1.36)	NS
multiple	0.75 (0.41,1.38)	NS
Free testosterone (148 pmol/l)		
age, BMI	1.21 (0.74,1.99)	NS
multiple	1.10 (0.61,1.99)	NS
SHBG (log unit)		
age, BMI	0.43 (0.12,1.55)	NS
multiple	0.50 (0.10,2.60)	NS
Oestrone (log unit)		
age, BMI	3.36 (0.55,20.6)	NS
multiple	2.13 (0.21,21.2)	NS
Oestradiol (29.54 pmol/l)		
age, BMI	0.74 (0.46,1.19)	NS
multiple	0.67 (0.36,1.26)	NS

NS, not significant ($p>0.1$); SD, standard deviation; CI, confidence interval; BMI, body mass index

Multiple adjustment for age, body mass index, systolic blood pressure, cigarette smoking (pack-years), serum low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides

[†] odds ratio for a one standard deviation increase (or a one unit increase on a log scale) in steroid sex hormone

Table 6.20. Multivariate logistic regression of steroid sex hormones on peripheral arterial disease in women

Hormone (SD or log unit) factors adjusted for	*Odds ratio (95% CI)*[†]		*p-value*
Total testosterone (0.45 nmol/l)			
age, BMI	0.79	(0.51,1.23)	NS
multiple	0.70	(0.41,1.17)	NS
Free testosterone (12.85 pmol/l)			
age, BMI	1.98	(0.63,2.51)	NS
multiple	0.77	(0.45,1.30)	NS
SHBG (log unit)			
age, BMI	0.59	(0.23,1.49)	NS
multiple	0.72	(0.24,2.15)	NS
Oestrone (log unit)			
age, BMI	0.69	(0.14,3.49)	NS
multiple	0.71	(0.12,4.43)	NS
Oestradiol (14.53 pmol/l)			
age, BMI	0.82	(0.53,1.28)	NS
multiple	0.79	(0.47,1.32)	NS

NS, not significant ($p > 0.1$), SD, standard deviation; CI, confidence interval; BMI, body mass index

Multiple adjustment for body mass index, systolic blood pressure, cigarette smoking, serum low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides

[†] odds ratio for a one standard deviation increase (or a one unit increase on a log scale) in steroid sex hormone

Chapter 7

Discussion

Glucose intolerance, steroid sex hormones and peripheral arterial disease

In this discussion chapter, methodological issues surrounding the analysis of the cross-sectional data in the Edinburgh Artery Study and the design of the case control study are considered first. The results of each study are then discussed in turn, with respect to the objectives presented in chapter 1.

7.1 Methodological considerations

7.1.1 Classification of peripheral arterial disease

In the Edinburgh Artery Study cross-sectional survey, responses to the World Health Organisation intermittent claudication questionnaire alone were used to identify subjects with symptomatic peripheral arterial disease. This is the most widely accepted method of detecting symptomatic peripheral arterial disease in epidemiological studies. Unfortunately, cases of intermittent claudication identified solely by the WHO questionnaire have been shown to include about one quarter of false positives when

compared to a clinician's diagnosis of claudication (Richard et al 1972). It is therefore likely that some of the subjects included in the disease group were biologically normal. One method of improving diagnostic specificity would have been to combine responses to the questionnaire with another marker of disease (for example, a low ankle brachial pressure index, as in the case control study), but this would have reduced overall sensitivity and so reduced the power of the study. Also, previous analysis had shown no difference in baseline risk factor profiles between subjects with WHO intermittent claudication alone and those with both claudication and a low ankle brachial pressure index (FGR Fowkes, personal communication).

To identify subjects with major asymptomatic peripheral arterial disease, a combination of the ankle brachial pressure index (ABPI) and reactive hyperaemia test was used. The ABPI is normally distributed within the general population, and it is generally accepted that as the ABPI falls, so the severity of the peripheral arterial disease increases. A cut-off point of 0.7 in ABPI was chosen as a single indicator of disease because, although arbitrary, results from clinical studies would suggest that few normal individuals have such a low level (Bernstein and Fronek 1982) and in the Edinburgh Artery Study population, subjects with such levels were well down in the normal distribution. However, false negative ABPI readings may result from rigid tibial arteries which prevent easy compression by the cuff (for example in association with calcification and severe diabetes), resulting in the use of cuff pressures which are higher than the actual intra-arterial pressure. Since abnormalities may be detected in such individuals when a stress is applied, the reactive hyperaemia test was used in conjunction with the ABPI to categorise subjects with asymptomatic disease when the

ABPI was between 0.9 and 0.7, thus improving diagnostic sensitivity. A drop in ankle systolic pressure of greater than 20% after occlusion was used since this would appear, in the clinical setting at least, to be valid in identifying patients with peripheral arterial disease (Baker 1978, Bernstein and Fronek 1982, Fowkes 1988a). When the result of the reactive hyperaemia test was used as an indicator of major asymptomatic disease disease on its own, a more severe cut-off point of 35% or greater was chosen. This categorisation of asymptomatic peripheral arterial disease has been used previously in the Edinburgh Artery Study, at which time validation of disease status was checked using duplex scanning. This confirmed, in a subsample of cases, the presence of significant atherosclerosis in those classified as asymptomatic disease but not in those classified as 'healthy' (Fowkes et al 1991).

In the case control study, subjects with symptomatic disease were also identified using the WHO intermittent claudication questionnaire. However, in order to minimise the number of false positives included in the case group, subjects with positive WHO intermittent claudication were included in the study only if they also had an ankle brachial pressure index of 0.9 or less in one leg. To identify cases with asymptomatic disease, an ankle brachial pressure index of 0.85 or less was taken as a single indicator of disease (since the reactive hyperaemia test was not repeated at the Edinburgh Artery Study five-year follow-up examination, this test could not be used to categorise subjects as in the cross-sectional study). A cut-off point of 0.85 in ABPI was chosen because this was the lowest possible level at which an adequate number of subjects could be identified for the study. The specificity of the ABPI in terms of detecting asymptomatic disease in subjects from the general population is

unknown. However, it is thought likely that (at least in subjects with relatively severe disease) this would be close to 100%, as reported for an ABPI <0.9 in the detection of angiogram-positive disease in hospital patients (Fowkes 1988a). Since specificity is likely to increase even further at lower levels of the index, the number of false positives included in the present study was therefore likely to be small.

To identify control subjects, an ABPI of 1.0 or greater in both legs was taken to indicate the absence of (asymptomatic) peripheral arterial disease. Since a cut-off value of less than 0.9 has been shown to have a sensitivity around 95%, and since this is likely to increase at higher cut-off values, the risk of including subjects with peripheral arterial disease in the control group (false negatives) was also small.

The most probable effect of including biologically normal subjects in the 'disease' or 'case' group and/or subjects with disease in the 'healthy' or control group would be to diminish associations between disease and risk factors. As such, this would not detract from any positive findings in the study.

7.1.2 Representativeness of study populations

The Edinburgh Artery Study population participating in the original cross-sectional survey was an age and sex-stratified, random sample of the general population of Edinburgh. Subjects were chosen from general practices serving the wide range of socio-economic and geographic areas present throughout the city of Edinburgh and the final social class structure of the study population was similar to that of the city

as a whole. Follow-up of a sample of non-responders indicated that there was no substantial bias in terms of age and sex between subjects agreeing to participate and those in the total selected population.

Comparison of age, sex and social class distributions indicated that subjects attending the 5-year follow-up examination (from which subjects for the case control study were selected) remained representative of the original study population. Cases agreeing to participate in the case control study were also similar to the total population of eligible cases identified, both in terms of age, sex and social class distribution and in terms of markers of peripheral arterial disease. In conclusion, both subjects participating in the cross-sectional study and cases participating in the case control study appeared to be representative of all persons, and all persons with disease respectively, in the general population. The results of both studies can therefore be extrapolated to the population in general with some confidence.

7.1.3 Bias

Bias is the systematic error in design or analysis of a study that results in incorrect estimates of risk factor association with disease. Case control studies are particularly susceptible to bias, although it can also affect the validity of results from other study types, including cross-sectional surveys. Bias can arise from differential reporting or recording of exposure information between study groups based on their disease status (observation bias) or, in case control studies, from the differential selection of cases or controls into the study on the basis of their exposure status (selection bias).

Selection bias is a particular problem when cases and controls are selected from two different populations. This invariably occurs in hospital-based case control studies, where persons with disease who have been treated at a particular hospital during a specified period are identified and matched with patients from another section of the hospital, relatives or the general population. In this situation, bias can arise from whatever factors lead an affected individual to utilise a particular health care facility. An advantage of the present population-based case control study was the ability to identify cases and controls from the same general population using subjects participating in the Edinburgh Artery Study, thus reducing the risk of selection bias. Since, in addition, response rates amongst cases and controls were similar and the exposure status of the subjects (their plasma insulin and sex hormone levels) was unknown at the time of selection, it is unlikely that selection bias had a major impact on the final results.

Observation bias occurs when there is differential reporting of exposure information between study groups based on their status as diseased or healthy. However, in the present studies, the majority of 'exposure' factors were haematological and personnel performing the assays for these factors were blinded to the subjects' disease status. Thus, blood samples were identified only by means of the subject study number, and laboratory personnel did not have access to the database which assigned disease status to each study number. In the case control study, batches of samples sent to participating laboratories contained samples from both cases and controls, and so any systematic variations in measurement or reporting of assay results over time should

have affected cases and controls equally. In addition, quality control procedures suggested that inter-batch variation was low.

It is possible that observation bias could have affected two of the other variables measured, namely smoking (reporting and/or interviewer bias) and waist hip ratio (measurement bias). Smoking status was based on the results of a smoking questionnaire. Interviewer bias (where the investigator may record smoking status more favourably for healthy subjects than for diseased subjects) was minimised by applying a self-administered questionnaire which clinic personnel only discussed occasionally with participants if there were difficulties. Although it is possible that subjects with peripheral arterial disease may have felt more guilty about smoking because of previous medical advice and may have underestimated their smoking compared with others (reporting bias), a good correlation between smoking levels and serum thiocyanate levels suggested that the smoking histories were probably reasonably accurate.

Waist hip ratio was measured by a single investigator (the author), to whom the disease status of the individual was potentially known. In an attempt to minimise measurement bias, waist and hip circumferences were measured according to a set protocol for every subject. Also, most clinics were attended by a combination of cases and controls, and on the day of the clinic, no documentation specifically identifying subjects as a case or a control was readily accessible. The author therefore did not automatically know the disease status of the individual, although this was sometimes apparent in general conversation.

7.2 Diabetes, cardiovascular risk factors and peripheral arterial disease

7.2.1 Prevalence of peripheral arterial disease in diabetes and impaired glucose tolerance

The prevalence of peripheral arterial disease in the Edinburgh Artery Study was 22.4% in diabetic subjects and 19.9% in subjects with impaired glucose tolerance. Differences in disease prevalence between the present and previous studies will reflect variations in definitions of peripheral arterial disease and diabetes (or impaired glucose tolerance), as well as different characteristics of the populations surveyed, such as age, sex and race. The present study included subjects with major asymptomatic peripheral arterial disease as well as subjects with intermittent claudication. It is not surprising, therefore, that the prevalence of peripheral arterial diseases was considerably higher than the 2% to 8% previously reported for intermittent claudication alone in diabetic populations (Nilsson et al 1967, Janka et al 1980, West et al 1983, Uusitupa et al 1990, Mohan et al 1996).

A more comparable study is a large-scale survey in England in which peripheral arterial disease was diagnosed according to an ankle brachial pressure index of 0.9 or less (Walters et al 1992). In general, the population characteristics of this study were also similar to the present study, in that disease was measured in a random sample of male and female type 2 diabetics from the general population, with a mean age of 67.7 years. The prevalence of peripheral arterial disease was found to be similar to that in the

Edinburgh Artery Study at 23.5%. These results suggest that peripheral arterial disease may affect between 1 in 4 and 1 in 5 type 2 diabetics in the United Kingdom.

As expected, the prevalence of peripheral arterial disease in subjects with diabetes or impaired glucose tolerance (20.6%) was greater than in those with normal glucose tolerance (12.5%). However, the degree of increased risk of peripheral arterial disease associated with diabetes or impaired glucose tolerance (1.45 times higher risk after age and sex adjustment), was rather less than the 2.5 to 6 times higher risks of disease previously reported in diabetic subjects (see Table 2.1). It is likely that this reflects two factors. Firstly, the inclusion of subjects with impaired glucose tolerance who had a slightly lower prevalence of peripheral arterial disease than frankly diabetic subjects. Secondly, a slightly higher than normal prevalence of peripheral arterial disease in the non-diabetic population, possibly due to a particularly poor risk factor profile in Scotland in terms of hypercholesterolaemia and cigarette smoking.

7.2.2 Risk factors for peripheral arterial disease in subjects with diabetes or impaired glucose tolerance

Consistent with the results of several previous studies (Schroll and Munck 1981, Kannel and McGhee 1985, Leng and Fowkes 1993), higher levels of cigarette smoking, systolic blood pressure, serum triglycerides, serum total cholesterol and serum low density lipoprotein cholesterol, and lower levels of serum high density lipoprotein cholesterol were found to be risk factors for peripheral arterial disease in normal glucose tolerant subjects. A similar array of factors appeared to increase the risk of

peripheral arterial disease in subjects with diabetes or impaired glucose tolerance, with levels of systolic blood pressure, plasma triglycerides and cigarette smoking particularly high in subjects with peripheral arterial disease compared with the 'healthy' group.

Most (Janka et al 1980, Paisey et al 1984, Kreines et al 1985, Kannel et al 1990, Uusitupa et al 1990, Mehler et al 1997, Al Zahrani et al 1997) but not all (West et al 1983, Walters et al 1992,) previous studies have also found a relationship between hypertension, hypertriglyceridaemia and smoking and the risk of peripheral arterial disease in diabetic subjects. Two studies found an association between hypertension and a low ankle brachial pressure index in diabetic subjects (Mehler et al 1997, Al Zahrani et al 1997), whilst in the University Group Diabetes program, baseline serum triglyceride was significantly related to the risk of intermittent claudication in men (Kreines et al 1985). In Finland, baseline systolic blood pressure, serum triglycerides and, in men, smoking were related to the five-year incidence of WHO intermittent claudication in newly-diagnosed type 2 diabetics (Uusitupa et al 1990). An association between both hypertension and hypertriglyceridaemia and the subsequent development of intermittent claudication in men and women with diabetes was also reported in the Framingham study (Kannel et al 1990). In a clinic-based sample of type 2 diabetic subjects, logistic regression also revealed a relationship between peripheral arterial disease and smoking (Paisey et al 1984).

In the present study, differences in serum total, low density and high density lipoprotein cholesterol between subjects with and without peripheral arterial disease did not reach statistical significance, possibly due to relatively small subject numbers. However,

other studies *have* shown associations between manifestations of peripheral arterial disease and raised serum total cholesterol and low density lipoprotein cholesterol and with reduced serum high density lipoprotein cholesterol in diabetic subjects (Kannel et al 1990, Uusitupa et al 1990, Maser et al 1991, Walters et al 1992).

Taken together, these results suggest that the same risk factors which are important in the development of peripheral arterial disease in normal glucose tolerant subjects are also important in diabetic subjects, with systolic blood pressure, serum triglycerides and cigarette smoking potentially having a particularly important role in diabetes.

7.2.3 Contribution of cardiovascular risk factors to the risk of peripheral arterial disease in diabetes

As expected (Laakso and Lehto 1998), subjects with diabetes or impaired glucose tolerance had a more detrimental cardiovascular risk factor profile compared with normal glucose tolerant subjects. In particular, systolic blood pressure and serum triglycerides were higher in glucose intolerant subjects, whether or not they also had peripheral arterial disease. The only exception was cigarette smoking; more subjects with diabetes or impaired glucose tolerance were never smokers and they had lower mean lifetime smoking. The Framingham study also found increased levels of cigarette smoking in male non-diabetic subjects compared with the male diabetic group, although women with diabetes tended to smoke more than those without diabetes (Kannel et al 1990).

Multivariate analysis indicated that raised serum triglycerides and elevated systolic blood pressure in subjects with diabetes or impaired glucose tolerance could explain, at least statistically, a major portion of the increased risk of peripheral arterial disease in these subjects. Adjustment for all of the other risk factors individually resulted in, if anything, only a slight reduction in the impact of diabetes on the likelihood of having peripheral arterial disease, while cigarette smoking *increased* the odds of diabetes or impaired glucose tolerance as a risk factor for disease (due to the higher percentage of never smokers in the diabetic/glucose intolerant group). In the only other previous study to attempt similar multivariate adjustment, the relative risk of peripheral arterial disease associated with baseline diabetic status was substantially reduced, but remained significantly elevated, after adjustment for age, serum cholesterol, smoking, systolic blood pressure and left ventricular hypertrophy. However, serum triglyceride levels were not included in this model (Kannel et al 1990).

There are several possible explanations for the results presented here. Firstly, since this was a cross-sectional study, the temporal relationship between altered risk factor levels and the development of peripheral arterial disease cannot be established and it is possible that raised systolic blood pressure and serum triglycerides were secondary to the disease. This possibility must be considered since previous prospective studies have failed to show a consistent relationship between elevated systolic blood pressure and the development of peripheral arterial disease, and, although most have found a univariate association between raised triglyceride levels and disease, this may be explained by a high correlation between triglyceride levels and cholesterol lipoproteins. A second explanation may be that some underlying phenomenon, such as insulin resistance

syndrome (Reaven 1988), was responsible for both the elevation in systolic blood pressure and serum triglycerides, and the higher prevalence of peripheral arterial disease. However, there may also be a direct causal relationship between raised systolic blood pressure and serum triglycerides and the development of peripheral arterial disease, in which case better control of these risk factors in diabetics should lead to a reduced incidence of disease; this can only be proven in future clinical trials.

Finally, this study considered only a limited number of those risk factors which could potentially contribute to the increased risk of peripheral arterial disease in diabetes. Although the odds ratio for peripheral arterial disease associated with diabetes became non-significant after adjustment for systolic blood pressure and serum triglycerides, the power of the study was limited by relatively small numbers of subjects, especially in the group with both diabetes or impaired glucose tolerance *and* peripheral arterial disease (the statistical significance of the odds ratio was affected by wide confidence intervals, and the absolute value of the ratio remained greater than 1.0). It is likely that factors other than systolic blood pressure and serum triglycerides contribute to the increased risk of peripheral arterial disease in subjects with diabetes. Indeed, the final risk of peripheral arterial disease in diabetes is likely to result from the net effect of a wide range of different risk factors. For example, diabetics are known to have pro-thrombotic states compared with non-diabetics; the additional influence of such factors on the increased risk of peripheral arterial disease in diabetes merits further investigation.

7.3 Hyperinsulinaemia and arterial disease

7.3.1 Univariate association between hyperinsulinaemia and peripheral arterial disease

In the case control study, plasma insulin levels one hour after an oral glucose load were significantly higher in subjects with symptomatic or severe asymptomatic peripheral arterial disease compared with 'healthy controls'. This is consistent with findings for coronary artery disease, where several large prospective studies found that raised plasma insulin levels were associated with an increased risk of disease in men (Pyörälä et al 1985, Fontbonne et al 1991, Yarnell et al 1994, Després et al 1996). It also confirms the findings of early studies on peripheral arterial disease, in which non-diabetic men with intermittent claudication had raised post-glucose plasma insulin levels compared with controls (Welborn 1966, Sloan et al 1970, Sorge et al 1976). In addition, associations between fasting plasma insulin levels and peripheral arterial disease, including the development of intermittent claudication, have been reported in diabetic subjects (Standl and Janka 1985, Uusitupa et al 1990). Since the insulin immunoassay in the present study did not cross-react with pro-insulin (as may have been the case in earlier studies), this study confirms that the active insulin metabolite is associated with disease.

Recently, the results of further studies on plasma insulin and peripheral arterial disease have been published, with generally inconsistent results (Table 7.1). In the Hoorn Study, neither fasting nor 2-hour post-glucose plasma insulin was associated

with the presence of peripheral arterial disease, as indicated by abnormal Doppler wave forms, an ABPI less than 0.9 or a history of prior vascular surgery (Beks et al 1995). However, the random population sample used in this study was stratified by glucose tolerance, such that it included a high proportion of subjects with diabetes and impaired glucose tolerance as well as normal glucose tolerant subjects; it is possible that other factors associated with each of these groups confounded any relationship between plasma insulin and disease. Three further studies considered predominantly asymptomatic disease in non-diabetic subjects (Laakso et al 1991, Jacob et al 1995, Kekäläinen et al 1996). No association was found between fasting or post-glucose insulin levels and the presence of femoral atherosclerosis at ultrasound examination (Laakso et al 1991, Kekäläien et al 1996), but non-diabetic patients with a low ankle brachial pressure index were found to have elevated insulin levels (Jacob et al 1995). In diabetic subjects, increased intima media thickness and arterial disease of the carotid and femoral arteries were associated with lower concentrations of fasting serum insulin (Elkeles et al 1996). The reasons for these apparently conflicting results are unclear, but may reflect differences in the methods used to detect peripheral arterial disease and the degree to which the compared, non-diseased study population was free from atherosclerotic disease.

Most previous studies on the relationship between plasma insulin and peripheral arterial disease have included only men, raising the question of whether insulin is also associated with risk of arterial disease in women. This is particularly interesting since diabetes is a two to three fold stronger risk factor for coronary artery disease in women than in men (Barrett-Connor et al 1991), and the same appears to be true for peripheral

arterial disease (Reunanen et al 1982, Uusitupa et al 1990). Of the three original large-scale prospective studies on coronary artery disease, the Helsinki Policeman Study (Pyörälä et al 1979, Pyörälä et al 1985), the Paris Prospective Study (Ducimetiere et al 1980, Fontbonne et al 1991) and the Busselton Study (Welborn and Wearne 1979, Cullen et al 1983), only one included women. In the Helsinki Policeman Study (Pyörälä et al 1985) and the Paris Prospective Study (Fontbonne et al 1991) raised fasting and/or post-glucose insulin levels were associated with the subsequent development of coronary artery disease in middle-aged men. Women were included in the Busselton Study, but the most recent analysis from this study indicated that hyperinsulinaemia did not affect coronary mortality in either sex (Cullen et al 1983). More recent prospective studies have also demonstrated an association between coronary artery disease and hyperinsulinaemia, but again these were confined to men (Yarnell et al 1994, Després et al 1996). Of only three cross-sectional studies to included both sexes, two found fasting (Rönnemaa et al 1991) or post-glucose (Mykkänen et al 1993) insulin levels to be indicators of coronary artery disease in both sexes. The third showed an excess coronary risk associated with hyperinsulinaemia in men only (Modan et al 1991).

Although women have been included more often in recent studies on peripheral arterial disease, results have not generally been reported separately for the two sexes. In the present study, mean post-glucose insulin levels were higher in both male and female cases than in sex-matched controls, although the difference in women did not quite reach statistical significance. However, there was no significant difference in the strength of the association between insulin and disease in men compared with women.

The most likely explanation for these results is that post-glucose insulin is associated with peripheral arterial disease in both sexes, and that lack of a significant difference in insulin levels between female cases and controls reflected a small sample size.

7.3.2 Multivariate association between hyperinsulinaemia and peripheral arterial disease

The present study considered a wide range of potentially confounding risk factors, including blood pressure, serum triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol. Detrimental levels of these risk factors are found to cluster in subjects with hyperinsulinaemia, possibly as part of the metabolic syndrome, syndrome X (Reaven 1988, Stout 1990), and an adverse effect of plasma insulin on such risk factors may explain some of the effect of insulin on disease. However, the present study showed, for the first time, that the association between insulin and peripheral arterial disease in non-diabetic subjects was independent of blood pressure and only partially dependent on serum triglycerides, serum low density lipoprotein cholesterol and serum high density lipoprotein cholesterol. The same independent relationship has been observed recently between insulin and coronary artery disease (Després et al 1996) and between hyperinsulinaemia and the incidence of intermittent claudication in diabetic subjects (Uusitupa et al 1990).

7.3.3 Biological explanation for association between hyperinsulinaemia and peripheral arterial disease

There are several possible explanations for the independent association found here between peripheral arterial disease and plasma insulin. Firstly, hyperinsulinaemia may cause atherosclerosis of the lower limbs either directly (via a direct pro-atherogenic effect on blood vessel walls), and/or indirectly via an adverse effect of high plasma insulin levels on cardiovascular risk factors not considered here. Thus, under experimental conditions, insulin stimulates proliferation and migration of arterial smooth muscle cells. It also stimulates cholesterol synthesis and low density lipoprotein binding in arterial smooth muscle cells and macrophages (Stout 1990). However, in addition to its association with blood pressure and dyslipidaemia, hyperinsulinaemia has been linked to adverse levels of other cardiovascular risk factors, including plasminogen activator inhibitor-1 (Elliott and Viberti 1993), and this could potentially mediate the effect of hyperinsulinaemia on arterial disease.

Secondly, some other factor, associated with hyperinsulinaemia, could be the important aetiological factor with insulin serving simply as a 'marker' for the underlying abnormality. A possible candidate for such an aetiological factor includes raised blood glucose levels. However, in the present study, the association between insulin and peripheral arterial disease remained significant after adjustment for blood glucose, suggesting that hyperinsulinaemia has an effect on disease independent of co-existent hyperglycaemia. Others have suggested that hyperinsulinaemia may act as a marker for insulin resistance, with the latter determining cardiovascular risk (Laakso et al 1991);

this theory received support when coronary artery disease was found to be directly associated with insulin resistance in men (Bressler et al 1992). However, in the present study, insulin resistance itself was not associated with peripheral arterial disease.

Finally, as with any cross-sectional or case control study, the possibility that hyperinsulinaemia was a secondary manifestation of existing vascular disease must be considered (Godsland and Stevenson 1995). Generalised atherosclerosis and reduced vasodilatory capacity in subjects with peripheral arterial disease may be associated with reduced glucose uptake into skeletal muscle and compensatory hyperinsulinaemia (Laakso et al 1990, Baron 1994). In addition, endothelial dysfunction caused by atherosclerosis may affect transport of insulin across capillary membranes (Godsland and Stevenson 1995), leading to elevated post-glucose insulin levels in the presence of normal fasting levels, as in the present study. A prospective cohort study will be required to ensure hyperinsulinaemia precedes the development of peripheral arterial disease.

7.3.4 Smoking, insulin and peripheral arterial disease

An association between smoking, a particularly strong risk factor for peripheral arterial disease (Powell 1991), and post-glucose insulin levels was also demonstrated. Furthermore, this association explained at least some of the effect of insulin on disease. Smoking has not traditionally been associated with hyperinsulinaemia and/or syndrome X, although a recent report found higher insulin levels and greater insulin resistance

associated with hypertriglyceridaemia and reduced high density lipoprotein cholesterol levels in 20 smokers than in 20 non-smokers (Facchini et al 1992). Fasting insulin levels were also raised in smokers and ex-smokers compared with non-smokers in 616 non-diabetic men from the general population (Rönnemaa et al 1996). This relationship was independent of other factors potentially affecting insulin sensitivity. It has been hypothesised that insulin resistance, hyperinsulinaemia and dyslipidaemia in cigarette smokers might be one mechanism by which smoking increases the risk of atherosclerotic disease (Facchini et al 1992). Although the present study confirmed a relationship between smoking and insulin levels and further, showed that this relationship was independent of other cardiovascular risk factors, neither insulin levels alone, nor in combination with high density lipoprotein cholesterol and triglyceride levels, accounted for the effect of smoking on disease.

7.4 Endogenous steroid sex hormones and peripheral arterial disease

The present case control study was the first epidemiological investigation into the relationship between endogenous steroid sex hormones and the risk of peripheral arterial disease. A wide range of sex hormones, as well as sex hormone-binding globulin was measured, using plasma which had been stored for only a limited time and subjects from the general population rather than hospitals. Despite this, there was no evidence to support the suggestion that plasma testosterone (total or free), sex hormone-binding globulin, oestradiol or oestrone were risk factors for peripheral

arterial disease in either men or women. The most important risk factors were systolic blood pressure, cigarette smoking, serum triglycerides, body mass index and waist hip ratio in men and smoking and reduced serum high density lipoprotein cholesterol in women.

7.4.1 Men

Several case control studies of endogenous hormones in men with coronary artery disease found higher oestrogen levels and lower testosterone levels in survivors of myocardial infarction compared with controls (Kalin and Zumoff 1990). Although in the present study plasma oestrone levels were slightly higher in male cases than in male controls, this relationship was only weakly significant and became non-significant after adjustment for age and body mass index. Obesity has been associated with altered sex hormone levels (in particular oestrogens) in previous studies, although these factors did not correlate in the present study, possibly due to the small number of obese subjects in the study sample. Obesity is also a potential risk factor for cardiovascular disease, and was therefore an important confounding factor. Neither mean plasma oestradiol nor total plasma testosterone levels were significantly different in men with peripheral arterial disease compared with controls. These results are consistent with those of prospective studies, in which none of the three sex hormones, oestradiol (Cauley et al 1987, Phillips et al 1988, Barrett-Connor and Khaw 1988, Eldrup et al 1989, Yarnell et al 1993), oestrone (Cauley et al 1987, Barrett-Connor and Khaw 1988, Eldrup et al 1989), or total testostosterone (Cauley et al 1987, Phillips et al 1988, Barrett-Connor and Khaw 1988, Yarnell et al 1993) predicted subsequent

myocardial infarction. It is likely that the altered hormone levels found in myocardial infarction survivors were a consequence of myocardial infarction, rather than a precursor. In our population, only 20% of male cases and 14% of female cases had a history of myocardial infarction and none of these events occurred within the year prior to hormone measurement.

More than 90% of total plasma testosterone circulates bound to proteins, predominantly sex hormone-binding globulin, and it is possible that only the free (bioavailable) testosterone fraction is important in determining cardiovascular risk. However, neither in the present study, nor in prospective studies of coronary artery disease, was free plasma testosterone associated with the presence or development of atherosclerotic disease (Cauley et al 1987, Barrett-Connor and Khaw 1988). More recently, it has been suggested that sex hormone-binding globulin itself might be a risk factor for cardiovascular disease, largely because of observed correlations between sex hormone-binding globulin and cardiovascular risk factors such as high density lipoprotein cholesterol and insulin (Pugeat et al 1995). However, the present study found no significant difference in the levels of sex hormone-binding globulin between men with and without peripheral arterial disease, consistent with previous case control (Hämäläinen et al 1987, Hautanen et al 1994) and prospective (Barrett-Connor and Khaw 1988) studies on sex hormone-binding concentration and coronary artery disease in men.

7.4.2 Women

Postmenopausal women taking pharmacological doses of exogenous estrogens reduce their risk of cardiovascular disease (Stampfer and Colditz 1991). However, there was no significant difference in endogenous oestrone or oestradiol levels between women with and without peripheral arterial disease in the present study, suggesting that physiological levels of estrogens may not be associated with disease. Correspondingly, no association has been found between circulating oestrone levels and prevalent heart disease (Cauley et al 1994), nor between oestrone or oestradiol and the development of symptomatic cardiovascular disease (Barrett-Connor and Goodman-Gruen 1995).

Neither testosterone (total or free), nor sex hormone-binding globulin levels differed between women with and without peripheral arterial disease in the present study. Previous information on endogenous testosterone levels and atherosclerotic disease in women is scarce. Although women with testosterone excess associated with chronic anovulation had an increased risk of myocardial infarction (La Vecchia et al 1987), in a single prospective study, neither total nor free testosterone predicted fatal cardiovascular disease in postmenopausal women (Barrett-Connor and Goodman-Gruen 1995). However, low sex hormone-binding globulin levels did increase the twelve year incidence of cardiovascular disease in postmenopausal women (Lapidus et al 1986).

An important consideration in any negative study is whether the study had sufficient power to detect a significant difference. With the given sample size and at a power of 80% (α=0.05), the present study could detect a difference in each sex hormone equivalent to approximately half a standard deviation of it's sex specific distribution (for example, a difference in total testosterone of 2.55 nmol/l in men and 0.27 nmol/l in women). It is possible that smaller differences might have been detected using larger subject numbers, but the physiological relevance of such small differences is questionable. It is also possible that the results could have been affected by the sensitivity of the hormone assays; in the laboratory, 18% and 22% of women were below the sensitivity range for oestradiol and total testosterone respectively, but no men were below the sensitivity range of any of the sex hormones and no women were below the sensitivity range for oestrone or sex hormone-binding globulin. Diurnal variation in hormone levels should not have affected the results since all samples were taken fasting at the same time in the morning.

The population studied in this investigation was relatively elderly (mean age 71 years). The fact that exogenous estrogens reduce cardiovascular risk in postmenopausal women suggests that exposure to sex hormones, even in later life, may be an important factor in determining disease. However, the latency period of cardiovascular disease is long, and it is also possible that any effects of endogenous sex hormones on disease are more important earlier in life, during the development phase of atherosclerosis. This is a particularly important consideration in women, since oestrogen levels fall substantially during the menopause. Although study of premenopausal women would be difficult due to their low risk of cardiovascular disease (necessitating prolonged

follow up) and difficulties in determining 'average' hormone concentrations during the menstrual cycle, such studies are necessary to demonstrate whether endogenous sex hormones are indeed important in the subsequent development of atherosclerotic disease.

7.5 Chapter summary

The work presented here was inspired by several observations described in the world literature on vascular disease, in particular, the higher prevalence of peripheral arterial disease in people with diabetes compared with non-diabetics and in men compared with women. Also the intense worldwide interest in the interaction between hormones and vascular disease and in the role of hyperinsulinaemia and sydrome X. The analysis subsequently undertaken on the Edinburgh Artery Study suggests three main findings: an important explanatory role for systolic blood pressure and serum triglycerides in the increased prevalence of peripheral arterial disease in diabetic subjects; a detrimental effect of hyperinsulinaemia on the development of peripheral arterial disease in non-diabetic subjects; and a lack of effect of endogenous steroid sex hormones on peripheral arterial disease in postmenopausal women or men.

Table 7.1. Plasma insulin and risk of peripheral arterial disease in epidemiological studies

Reference	Type of study	Population (no.)	Age (years)	Definition of peripheral arterial disease	Insulin levels	Association with PAD	Comment
Welborn et al 1966	Case control	Cases: Non-diabetic hospital patients admitted for angiography (7) Controls: Non-obese healthy volunteers (45)	Mean 51 Mean 31	IC (vascular occlusion confirmed at angiography)	Fasting 1-hour 2-hour	- + -	Small study with no matching of cases and controls
Sloan et al 1970	Case control	Cases: Non-diabetic hospital patients, male (51) Controls: Hospital controls, male (47)	Mean 54 Mean 50	IC (atherosclerosis confirmed at arteriography)	Fasting 1-hour 2-hour	- + +	No matching or multivariate adjustment
Sorge et al 1976	Case control	Cases: Non-diabetic hospital patients, men and women (65) Controls: Hospital outpatients (89)	Mean 60 Mean 57	Surgically treated or identified at arteriography	Fasting 1-hour 2-hour	- + +	Cases and controls matched for age, glucose tolerance and weight
Uusitupa et al 1990	Prospective	Men and women with newly-diagnosed type 2 diabetes (133) Non-diabetic men and women (144)	45-64	Incidence of IC	Fasting Fasting	+ -	'Trend' to raised 1 and 2-hour post-glucose insulin in diabetics with IC
Laakso et al 1991	Case control	Cases: Nonobese, non-diabetic men from general population (30) Controls: Population controls (13)	Mean 55 Mean 53	Asymptomatic athero-sclerosis of femoral or carotid arteries (at ultrasound)	Fasting 1-hour 2-hour	- - -	Small study - matched for age and BMI
Beks et al 1995	Cross sectional	Random population sample of diabetic and non-diabetic men and women (631)	50-74	ABPI, Doppler wave-forms, vascular surgery	Fasting 2-hour	- -	Population sample stratified by glucose tolerance
Jacob et al 1995	Cross sectional	Patients attending general medical practice (59)	Unknown	ABPI<0.8 or 0.8-1.0	Fasting	+	Matching for age and weight
Elkeles et al 1996	Cross sectional	Type 2 diabetic men and women (192)	35-66	Carotid IMT, USS of carotid and femoral arteries	Fasting	Inverse	Multiple regression analysis
Kekäläinen et al 1996	Cross sectional	Non-diabetic men and women from general population (87)	39-56	USS of femoral arteries	Fasting 2-hour	- -	Only 33 subjects with femoral plaques

IC, intermittent claudication; ABPI, ankle brachial pressure index; IMT, intimal medial thickness; USS, ultrasound scan

Chapter 8

Conclusions and recommendations

In this chapter, the principal conclusions of the work presented in the thesis are summarised and recommendations for further research in this area are listed.

8.1 Conclusions

8.1.1 Diabetes and peripheral arterial disease

Subjects with diabetes or impaired glucose tolerance from the general population have a higher prevalence of symptomatic and asymptomatic peripheral arterial disease compared with normal glucose tolerant subjects. In people with diabetes, systolic hypertension, hypertriglyceridaemia and cigarette smoking may be particularly important risk factors for the development of peripheral arterial disease. Indeed, raised levels of serum triglycerides and systolic blood pressure in subjects with diabetes or impaired glucose tolerance may explain a major portion of their increased risk of peripheral arterial disease compared with normal glucose tolerant subjects.

8.1.2 Hyperinsulinaemia and peripheral arterial disease

Peripheral arterial disease is associated with raised plasma insulin levels and it is most likely that this is the case for both men and women. The relationship between plasma insulin and peripheral arterial disease is independent of other cardiovascular risk factors associated with hyperinsulinaemia, including blood pressure, serum low density and high density lipoprotein cholesterol, serum triglycerides, and blood glucose, suggesting that insulin may have a direct effect on atherogenesis. However, smokers appear to be hyperinsulinaemic compared with non-smokers and this may explain some of the relationship between insulin and peripheral arterial disease. This latter finding also raises the intriguing possibility that raised plasma insulin levels could contribute to the detrimental effect of cigarette smoking on peripheral arterial disease.

8.1.3 Steroid sex hormones and peripheral arterial disease

Mean plasma levels of a range of steroid sex hormones, including total and free testosterone, sex hormone-binding globulin, oestradiol and oestrone, are similar in elderly men and postmenopausal women with and without peripheral arterial disease. This does not support the hypothesis that endogenous sex hormone levels are important in determining risk of atherosclerotic disease in either sex. However, neither does it preclude a role for endogenous steroid sex hormones in the development of atherosclerosis in younger men or premenopausal women.

8.1.4 Aetiology of peripheral arterial disease

Research into peripheral arterial disease has increased recently. However, there is still much to be learned about the aetiology of this condition, especially in comparison with coronary artery disease. The findings described here provide some insight into the importance of glucose intolerance, hyperinsulinaemia and endogenous steroid sex hormones in the aetiology of a condition which, ultimately, is likely to result from a web of interactions between a large number of risk factors, both genetic and environmental. The on-going challenge is to unravel this web.

8.2 Recommendations for future research

1. A prospective cohort study is required to confirm the temporal relationship between the risk factors identified here and the development of peripheral arterial disease in subjects with diabetes. Unfortunately, the Edinburgh Artery Study population, whilst being followed up prospectively, is likely to contain too few diabetic subjects to enable such an analysis. However, the cross-sectional data already available *can* be analysed to investigate the impact of a wider range of risk factors than was included here on the higher prevalence of peripheral arterial disease in diabetic populations. This includes haemostatic and rheological factors such as plasma fibrinogen and plasminogen activator inhibitor which are known to be raised in people with diabetes.

2. Within randomised controlled trials aimed at reducing the incidence of macrovascular complications in general (including coronary artery disease) in type 2 diabetes, it should be possible to investigate the effects of lowering systolic blood pressure and serum triglycerides on the risk of peripheral arterial disease (in particular, to determine whether strict regulation of these factors can reduce the risk of peripheral arterial disease to levels comparable with those of non-diabetic subjects).

3. Prospective studies are also required to establish the temporal relationship between hyperinsulinaemia and the development of peripheral arterial disease. Such studies should include both men and women in large enough numbers to allow separate analysis according to sex. Careful consideration should be given to the methods used to detect the development of peripheral arterial disease and to the measurement of the wide range of potentially confounding cardiovascular risk factors, particularly those linked with syndrome X.

4. Given the preponderance of studies on plasma insulin and coronary artery disease, further studies are required on the relationship between insulin and other forms of arterial disease, such as cerebrovascular disease and measures of asymptomatic disease such as intimal medial thickening.

5. Further investigation is required into the significance of the relationship between cigarette smoking and plasma insulin levels and, in particular, whether raised insulin levels might mediate some of the effect of cigarette smoking on the development of arterial disease.

6. Further prospective studies are required to investigate the impact of endogenous steroid sex hormones on arterial disease in younger populations. In particular, it would be important to determine whether endogenous oestrogen levels in pre-menopausal women influence the development of arterial disease later in life.

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Appendix I. Edinburgh Artery Study baseline questionnaire

E D I N B U R G H A R T E R Y S T U D Y

QUESTIONNAIRE

THE INFORMATION IN THIS QUESTIONNAIRE IS HIGHLY CONFIDENTIAL AND IS PART OF A MEDICAL RESEARCH STUDY

The information you give in this personal health record will be treated as strictly confidential and will be available only to your own doctor and the study team. The results of the research will appear only in the form of general statistics from which it will be impossible to identify you as an individual.

Please complete the following:

SURNAME: .....

FORENAMES: .....

DATE: .....

If you have any difficulties in answering some questions you will have a chance to discuss these later with a member of the study team.

THANK YOU FOR YOUR CO-OPERATION IN THIS STUDY. THE FINDINGS WILL HELP TO IMPROVE HEALTH IN SCOTLAND.

IT IS IMPORTANT TO ANSWER ALL THE QUESTIONS CAREFULLY. PLEASE TAKE YOUR TIME.

There is some evidence of a relationship between health and other factors such as exercise, occupation, education, diet etc. In order to compare our data with national figures and other research work, we are interested to have the following details about yourself.

PERSONAL HISTORY

1. Please tick one box:

Male	Female
☐ 1	☐ 2

2. Enter your date of birth:

Day	Month	Year
☐☐	☐☐	☐☐

3. Please tick the box showing your present marital status:

Married (or equivalent)	☐ 1
Single	☐ 2
Widowed	☐ 3
Divorced or separated	☐ 4

EDUCATION

4. What is the HIGHEST level of education you and your spouse or ex-spouse have completed? Please tick boxes as appropriate.

	Yourself	Spouse or Ex-spouse
University/college degree course	☐ 1	☐ 1
Other professional or technical qualification after leaving school	☐ 2	☐ 2
Secondary School	☐ 3	☐ 3
Primary School	☐ 4	☐ 4

PAID EMPLOYMENT

5. What is your employment status at the moment? Please tick boxes as appropriate.

Employed, full-time	☐ 1
Employed, part-time	☐ 2
Unemployed	☐ 3
Retired	☐ 4
A housewife (full-time)	☐ 5
Other, please specify	☐ 6

Please complete questions 6 and 7 as appropriate for yourself and your spouse or ex-spouse.

6. <u>YOURSELF</u> <u>YOUR SPOUSE or EX-SPOUSE</u>

(a) Please give the name of your present job and describe what you do as fully as possible. If unemployed or retired, do not complete this question, BUT PROCEED TO QUESTION 7.

....

....

....

....

(b) What business or industry is this in?

....

(c) In this job are you?

self-employed	☐	foreman	☐	self-employed	☐	foreman	☐
manager	☐	other	☐	manager	☐	other	☐

(d) In this job do you supervise/employ?

25 or more people	☐	25 or more people	☐
fewer than 25 people	☐	fewer than 25 people	☐
no-one	☐	no-one	☐

7. YOURSELF | YOUR SPOUSE or EX-SPOUSE

(a) Please give the name of the job you have done for the longest period of your life, and describe what you did as fully as possible. (If the answer is the same as in Question 6 above, write SAME)

....

....

....

....

(b) What business or industry was this in?

....

(c) In this job were you?

self-employed	☐	foreman	☐	self-employed	☐	foreman	☐
manager	☐	other employee	☐	manager	☐	other employee	☐

(d) In this job did you supervise/employ?

25 or more people	☐	25 or more people	☐
fewer than 25 people	☐	fewer than 25 people	☐
no-one	☐	no-one	☐

FOR OFFICE USE ONLY

S.C.

SMOKING

Smoking has been linked with many health problems. It is important that you answer the following section as accurately as possible. Please tick appropriate boxes.

8(a) Do you smoke at present? Yes ☐ No ☐
IF NO, PROCEED TO QUESTION 8(f)

(b) What do you usually smoke now?

cigarettes	Yes ☐	No ☐
pipe	Yes ☐	No ☐
cigars	Yes ☐	No ☐

(c) How many do you usually smoke now?

cigarettes per day	.....	cigarettes
oz. tobacco per week	.....	oz.
cigars per week	.....	cigars

(d) For how many years during your life have you smoked cigarettes? ..... years

(e) How many cigarettes have you smoked on average per day during the period you have smoked? ..... cigarettes
NOW PROCEED TO QUESTION 8(k)

(f) Have you ever smoked regularly? Yes ☐ No ☐
IF NO, PROCEED TO QUESTION 8(k)

(g) What did you usually smoke?

cigarettes	Yes ☐	No ☐
pipe	Yes ☐	No ☐
cigars	Yes ☐	No ☐

(h) How much did you smoke on average while you were a smoker?

cigarettes per day	.....	cigarettes
oz. tobacco per week	.....	oz.
cigars per week	.....	cigars

(i) For how many years did you smoke cigarettes? ..... years

(j) If you smoked cigarettes, how long is it since you finally gave up? ..... years .....months

(k) Is any other member of your household a smoker? Yes ☐ No ☐

MEDICAL HISTORY

We should now like to ask you questions about your health, illnesses you have had in the past, and how you are feeling now. Please tick appropriate boxes.

9. Have you ever been told by a doctor that you have or have had any of the following?

	Yes	No
Hardening of the arteries in the legs	☐	☐
Angina	☐	☐
Heart attack (coronary thrombosis, myocardial infarction)	☐	☐
High blood pressure	☐	☐
Stroke	☐	☐
Diabetes (sugar disease)	☐	☐
Bronchitis	☐	☐
Tuberculosis	☐	☐
Asthma	☐	☐

10. Are you on any regular medical treatment from a doctor as follows?

	Yes	No
Drugs to lower blood pressure	☐	☐
Diuretics (water tablets)	☐	☐
Insulin injections	☐	☐
Tablets for diabetes	☐	☐
Other treatments? Give names if possible.	☐	☐

.....

.....

.....

.....

CHEST PAIN

11(a) Do you ever get pain or discomfort in your chest?
IF NO, PROCEED TO QUESTION 12

Yes ☐ No ☐

(b) Do you get this pain or discomfort when you walk uphill or hurry?
IF NO, PROCEED TO QUESTION 11g

Yes ☐ No ☐

(c) Do you get it when you walk at an ordinary pace on the level?

Yes ☐ No ☐

(d) When you get any pain or discomfort in your chest what do you do?

Stop ☐

Slow down ☐

Continue at the same pace ☐

(e) Does it go away when you stand still or sit down?

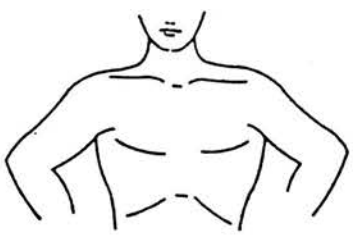
Yes ☐ No ☐

(f) How soon?

10 minutes or less Yes ☐ No ☐

more than 10 minutes Yes ☐ No ☐

(g) Where do you get this pain or discomfort? Mark the place(s) with 'X' on the diagram.

RIGHT  LEFT

12(a) Have you ever had a severe pain across the front of your chest lasting for half an hour or more?

Yes ☐ No ☐

(b) What was the cause? .....

FOR OFFICE USE ONLY A: GRADE MI:

COUGH

13(a) Do you usually cough several times first thing in the morning in the winter? (Ignore clearing throat or single cough)

Yes ☐ No ☐

(b) Do you usually cough during the day or night in winter? (Ignore the occasional cough)

Yes ☐ No ☐

(c) If yes to (a) or (b), do you cough on most days for at least three months each winter?

Yes ☐ No ☐

PHLEGM (SPIT)

14(a) Do you usually bring up any phlegm (spit) from your chest first thing in the morning in the winter?

Yes ☐ No ☐

(b) Do you usually bring up any phlegm from your chest during the day, or at night, in the winter?

Yes ☐ No ☐

(c) If yes to (a) or (b), do you bring up phlegm like this on most days for as much as three months each year?

Yes ☐ No ☐

BREATHLESSNESS

15(a) Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?

IF NO, GO TO QUESTION 16

Yes ☐ No ☐

(b) Do you get short of breath walking with other people of your own age on level ground?

Yes ☐ No ☐

(c) Do you have to stop for breath when walking at your own pace on level ground?

Yes ☐ No ☐

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C

P

B GRADE

WHEEZING

16(a) Have you had attacks of wheezing or whistling in your chest at any time in the last 12 months?

Yes ☐ No ☐

(b) Have you ever had attacks of shortness of breath with wheezing?

Yes ☐ No ☐

(c) If yes to (b), is/was your breathing absolutely normal between attacks?

Yes ☐ No ☐

(d) Have you at any time in the last 12 months been woken at night by an attack of shortness of breath?

Yes ☐ No ☐

LEG PAIN

17(a) Do you get a pain in either leg on walking?
IF NO, GO TO QUESTION 18

Yes ☐ No ☐

(b) Does this pain ever begin when you are standing still or sitting?

Yes ☐ No ☐

(c) Do you get this pain in your calf (or calves)?

Yes ☐ No ☐

(d) Do you get it when you walk uphill or hurry?

Yes ☐ No ☐

(e) Do you get it when you walk at an ordinary pace on the level?

Yes ☐ No ☐

(f) Does the pain ever disappear while you are still walking?

Yes ☐ No ☐

(g) What do you do if you get it when you are walking?

Stop 1 ☐

Slow down 2 ☐

Continue at same pace 3 ☐

(h) What happens to it if you stand still?

Usually continues for more than 10 minutes 1 ☐

Usually disappears in 10 minutes or less 2 ☐

18. Have you ever had surgery on the arteries of your legs other than for varicose veins?

Yes ☐ No ☐

Please specify .....

19. Have you ever had surgery to remove

toes? Yes ☐ No ☐

leg below the knee? Yes ☐ No ☐

leg above the knee? Yes ☐ No ☐

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I.C. GRADE

OTHER MEMBERS OF YOUR FAMILY

20. Please tick the appropriate boxes for other members of your family if they have been diagnosed as having any of the illnesses below:

Illnesses	Father	Mother	Any brother or sister	Any son or daughter
Angina	☐	☐	☐	☐
Stroke	☐	☐	☐	☐
High blood cholesterol level	☐	☐	☐	☐
Diabetes (sugar disease)	☐	☐	☐	☐
Hardening of the arteries in the leg/claudication	☐	☐	☐	☐
Thrombosis/embolism	☐	☐	☐	☐
High blood pressure	☐	☐	☐	☐
Heart attack	☐	☐	☐	☐
If died from heart attack, at what age?	....Yrs	....Yrs	....Yrs	....Yrs

PHYSICAL ACTIVITY

The following section gives examples of the sort of activities you might do or may have done REGULARLY.

LIGHT activity	MODERATE activity	STRENUOUS activity
Ballroom dancing	Badminton	Basketball
Bowling	Cricket	Competitive cycling
Light do-it-yourself	Cycling (include to and	Competitive swimming
Light gardening	from work,to shops etc)	Competitive running
Horse riding	Heavy do-it-yourself	Field sports (such as
Sailing	Golf	rugby, soccer, hockey)
Walking (include to and	Jogging	Training for strenuous
from work,to shops etc)	Swimming	sport
Yoga	Tennis	Squash
And other activities of similar intensity. Please specify others you have done.	And other activities of similar intensity. Please specify others you have done.	And other activities of similar intensity. Please specify others you have done.
.....	.....	.....
.....	.....	.....

21. In a typical week during the last year, on how many occasions would you take part FOR MORE THAN 20 MINUTES EACH TIME:
Insert'None'if appropriate

in LIGHT physical activity?	in summer	..... times
	in winter	..... times
in MODERATE physical activity?	in summer	..... times
	in winter	..... times
in STRENUOUS physical activity?	in summer	..... times
	in winter	..... times

22. In a typical week, when you were 35-45 years old, on how many occasions would you take part, FOR MORE THAN 20 MINUTES EACH TIME:
Insert 'None' if appropriate

in LIGHT physical activity?	in summer	..... times
	in winter	..... times
in MODERATE physical activity?	in summer	..... times
	in winter	..... times
in STRENUOUS physical activity?	in summer	..... times
	in winter	..... times

23. Which of the following best describes your daily work or other daytime activity at the present time?
Please tick one box only.

I am usually sitting during the day and do not walk about much	☐	eg. office workers, drivers
I stand or walk about quite a lot during the day, but do not have to carry or lift things very often	☐	eg. housewives, shop assistants
I usually lift or carry light loads and have to climb stairs and/or hills often	☐	eg. postmen, packers
I do heavy work and carry heavy loads	☐	eg. building, mining workers, agricultural workers

24. Which of the following best described your daily work or other daytime activity WHEN YOU WERE 35-45 YEARS OLD?

I usually sat during the day and did not walk about much	☐	eg. office workers, drivers
I stood or walked about quite a lot during the day, but did not have to carry or lift things very often	☐	eg. housewives, shop assistants
I usually lifted or carried light loads and had to climb stairs and/or hills often	☐	eg. postmen, packers
I did heavy work and carried heavy loads	☐	eg. building, mining heavy workers, agricultural workers

LIFE STYLE AND ATTITUDES

25. Each of us belongs somewhere along the line between two extremes. For example, most of us are neither the most competitive nor the least competitive person we know. What we would like you to do is to make a vertical line where you think you belong between these two extremes.

For example:

Always tidy ——————|—— Never tidy

(a)	Never late	_____	Casual about appointments
(b)	Not competitive	_____	Very competitive
(c)	Anticipate what others are going to say (nod, interrupt, finish for them)	_____	Good listener, hear others out
(d)	Always rushed	_____	Never feel rushed, even under pressure
(e)	Can wait patiently	_____	Impatient when waiting
(f)	Go "all out"	_____	Casual
(g)	Take things one at a time	_____	Try to do many things at once, think about what to do next
(h)	Emphatic in speech (may pound desk or chair))	_____	Slow, deliberate talker
(i)	Have wanted good job recognised by others	_____	Only care about satisfying myself no matter what others may think
(j)	Fast (eating, walking etc.)	_____	Slow doing things
(k)	Easy going	_____	Hard driving
(l)	Hide feelings	_____	Express feelings
(m)	Many interests	_____	Few interests (outside work)
(n)	Am/was satisfied with job	_____	Ambitious

26. The next section contains descriptions of how you may have felt, thought, or acted during most of your life. Below each statement there are four words or phrases; choose the one which best describes you for most of your life and draw a circle round it.

EXAMPLE

(a) I have enjoyed being with other people.

Nearly always (Often) Seldom Never

The first example would mean that most of your life you have often enjoyed being with other people.

(1) I would have liked to get my own back on someone.

Very often Often Seldom Never

(2) I have been content to act in a very humble way.

Never Seldom Often Nearly always

(3) I have thought that people will tell the truth, even if it gets them into trouble.

Nearly always Often Seldom Never

(4) I have felt as capable as other people.

Never Seldom Often Nearly always

(5) When I've wanted to have a row with someone, I have done so.

Nearly always Often Seldom Never

(6) I have preferred to take a lot of advice before doing anything.

Never Seldom Often Nearly always

(7) I have felt like telling people to go to blazes.

Nearly always Often Seldom Never

(8) When in a group I have been content to be led.

Never Seldom Often Nearly always

(9) When someone has been particularly helpful, I've wondered what real reason lay behind it.

Nearly always Often Seldom Never

(10) I have had confidence in myself.

Never Seldom Often Nearly always

(11) When I've disliked someone, I have shown it.

Nearly always Often Seldom Never

(12) I have wanted plenty of support from people.

Never Seldom Often Nearly always

(13) I have felt the urge to smash things.

Very often Often Seldom Never

(14) I have been content to be dominated by someone else.

Never Seldom Often Nearly always

(15) I have believed that people are pretty reliable.

Nearly always Often Seldom Never

(16) I have been very unsure of myself.

Never Seldom Often Nearly always

(17) When I've been angry with someone, I've bottled it up.

Nearly always Often Seldom Never

(18) I have liked to be told what needs doing.

Never Seldom Often Nearly always

(19) I have wanted to give someone a piece of my mind.

Very often Often Seldom Never

(20) I have preferred to let people have their own way.

Never Seldom Often Nearly always

(21) I have felt that people would tell lies to get ahead.

Nearly always Often Seldom Never

(22) I have given up doing something because I thought too little of my own ability.

Never Seldom Often Very Often

(23) Even when crossed, I've let people get away with it.

Nearly always Often Seldom Never

(24) I have been content to lean on other people for emotional support.

Never Seldom Often Nearly always

(25) I would have liked to pick a quarrel with someone.

Very often Often Seldom Never

(26) I have been happy to play second fiddle.

Never Seldom Often Nearly always

(27) I have felt that people are out for what they can get.

Nearly always Often Seldom Never

(28) I have felt that even when difficulties were piling up I would overcome them.

Never Seldom Often Very often

(29) When I've thought I was justified in losing my temper, I have done so in no uncertain terms.

Very often Often Seldom Never

(30) I have preferred to find out for myself what's to be done.

Never Seldom Often Nearly always

(31) I have felt like blaming others when things have gone wrong.

Nearly always Often Seldom Never

(32) I have preferred to stay in the background.

Never Seldom Often Nearly always

(33) I have thought one can safely trust people.

Nearly always Often Seldom Never

(34) I have felt pretty useless.

Never Seldom Often Nearly always

(35) When I've felt like blaming someone to their face for something that has gone wrong, I have done so.

Nearly always Often Seldom Never

(36) I have needed a lot of help from other people.

Never Seldom Often Very often

Appendix II. Clinic invitation letter (men and women)

[Title] [Initial] [Surname]
[Address 1]
[Address 2]
[Post Code]

[Date]

Dear [Title] [Surname]

Thank you for attending the Edinburgh Artery Study examination last year. Following our telephone conversation today, I am enclosing the details of a further examination which we are currently undertaking to help us find out more about the causes of arterial disease.

If you are willing to attend our research clinic this year, you would need to fast for twelve hours prior to your appointment. We would then take a blood sample and give you a drink of 'Lucozade', followed by a further blood sample after one hour. Refreshments will be provided at the end of the examination.

> I have arranged an appointment for you on **[Day] [Date]** at **9.15 am** in the **'Edinburgh Vein Study' rooms, Medical School Quadrangle, Teviot Place** (A map is enclosed).

I would be grateful if you could return the enclosed form, confirming whether or not you are able to attend on this date. If you have any queries about the study, please telephone me at the number above,

With many thanks for your continued cooperation,

Dr Jackie Price
Clinical Research Fellow

Please note: It is important that you have nothing to eat or drink (except for water) for the twelve hours preceding your appointment, ie. after 9 pm on [Date].

PLEASE COMPLETE THE FOLLOWING

SECTION A

	YES	NO
Do you presently take Hormone Replacement Therapy	☐	☐

If **NO**, continue to section B

If **YES**, it is not possible for you to be included in the present study. Please simply **return** this form and **ignore** both the appointment and section B of this form. (You will of course continue to be included in the Edinburgh Artery Study as before)

SECTION B

(tick only one box here)

1) I will be attending the clinic on
[Day] [Date] at 9.15am ☐

OR 2) I am unable to attend on [Date]
Please send me another appointment ☐

OR 3) I am NOT willing to attend for the
blood tests described above ☐

NAME .....

THANK YOU FOR COMPLETING THIS FORM
PLEASE RETURN IT IN THE PRE-PAID ENVELOPE

PLEASE COMPLETE THE FOLLOWING

(tick only one box)

1) I will be attending the clinic on
[Day] [Date] at 9.15am ☐

OR 2) I am unable to attend on [Date]
Please send me another appointment ☐

OR 3) I am NOT willing to attend for the
blood tests described above ☐

NAME .....

THANK YOU FOR COMPLETING THIS FORM
PLEASE RETURN IT IN THE PRE-PAID ENVELOPE

CASE CONTROL STUDY
Data collection form

Subject Name ..... Date ..... Subject No. .....

Has subject fasted? YES/NO (If NO, new appointment date.....)

Is subject on HRT? YES/NO

Is subject diabetic YES/NO (If YES, treatment.....)

Consent form signed? YES/NO

Time of Blood Sample 1 .....

Time of OGTT .....

Time of Blood Sample 2 .....

Waist circumference ..... cm Hip Circumference ..... cm

Are all microtubes filled? YES/NO

If NO, give details .....

✂.....

TRAVELLING EXPENSES

Subject Name: ..... Date: .....

Transport: ..... Cost: .....

Subject Signature: ..... Researcher Signature: .....

Subject No .....

CONSENT FORM

AETIOLOGY OF PERIPHERAL ARTERIAL DISEASE: A CASE CONTROL STUDY

Purpose of the Study

The purpose of this study is to obtain further information on constituents of the blood which may predispose people to developing narrowing of the arteries in the legs. It is also intended to look at how these factors differ between men and women. The ultimate aim is to improve disease prevention and treatment.

Research Examination

A blood sample will be taken and you will be given a sweet drink. A further blood test will be taken after one hour. Your waist and hip circumferences will also be measured.

Consent Agreement

I understand the purpose of this research which has been fully explained to me by a member of the research team. The study has been given ethical approval by the Medicine/Clinical Oncology Research ethics Sub-Comittee of the Lothian Health Board.

I give my consent to the research team carrying out the medical investigations on me as described, although I can withdraw from the examination at any point if I so wish.

NAME .....

ADDRESS .....

.....

.....

SIGNATURE ..... **DATE** .....

SIGNATURE OF RESEARCHER .....

CASE CONTROL STUDY
Venepuncture form

Patient Name ..... Date .....

Recorder: 1 J.P. 2 E.K. 3 F.S.

1. Has patient had hepatitis or jaundice suspicious of hepatitis? YES/NO
2. Has patient had childhood jaundice? YES/NO
3. Is patient known HIV positive? YES/NO
4. Was venepuncture normal? YES/NO
5. Was venepuncture difficult/slow? YES/NO
6. Was venepuncture not possible? YES/NO
7. How much blood was obtained?

   First blood test .....ml

   Second blood test .....ml

8. Other comments

   .....

   .....

Published papers

1. MacGregor AS, **Price JF**, Hau CM, Lee AJ, Carson MN, Fowkes FGR. The role of systolic blood pressure and plasma triglycerides in diabetic peripheral arterial disease: The Edinburgh Artery Study. Diabetes Care 1999;22:453-8.

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2. **Price JF**, Lee AJ, Fowkes FGR. Hyperinsulinaemia: a risk factor for peripheral arterial disease in the non-diabetic general population. J Cardiovasc Risk 1996;3:501-5.

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3. **Price JF**, Lee AJ, Fowkes FGR. Steroid sex hormones and peripheral arterial disease in the Edinburgh Artery Study. Steroids 1997;62:789-94.

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Role of Systolic Blood Pressure and Plasma Triglycerides in Diabetic Peripheral Arterial Disease

The Edinburgh Artery Study

ANDREW S. MACGREGOR, MB, CHB
JACQUELINE F. PRICE, MB, CHB
CATHRYN M. HAU, MSC
AMANDA J. LEE, PHD
MAGGIE N. CARSON, MSC
F. GERALD R. FOWKES, PHD

OBJECTIVE — To determine the risk factors for peripheral arterial disease (PAD) in a diabetic population and to examine whether different levels of these risk factors might explain why diabetic subjects have an increased risk of PAD compared with normal glucose tolerance subjects.

RESEARCH DESIGN AND METHODS — There were 1,592 men and women aged 55–74 years selected at random from the age-sex registers of 11 general practices in Edinburgh, Scotland. Subjects underwent a comprehensive medical examination, including assessment for PAD (intermittent claudication on World Health Organization questionnaire or major asymptomatic disease on noninvasive testing) and a glucose tolerance test.

RESULTS — Of the subjects, 288 (18.7%) were found to have diabetes or impaired glucose tolerance (IGT). The prevalence of PAD was greater in those with diabetes/IGT (20.6%) compared with those with normal glucose tolerance (12.5%) (odds ratio [OR] 1.64, 95% CI 1.17–2.31). Among the diabetes/IGT group, mean levels of smoking, systolic blood pressure, and triglycerides were higher in subjects with PAD than in those without PAD ($P \leq 0.05$). Mean levels of systolic blood pressure and plasma triglycerides were also higher in diabetic subjects than in nondiabetic subjects with PAD ($P \leq 0.05$). In multivariate analysis, those with diabetes/IGT no longer had a significantly higher risk of PAD after adjusting separately for systolic blood pressure (OR 1.22, 95% CI 0.85–1.73) and plasma triglycerides (OR 1.26, 95% CI 0.89–1.79). Simultaneous adjustment for both systolic blood pressure and triglycerides reduced the risk of PAD among diabetic subjects to 1.11 (95% CI 0.78–1.58).

CONCLUSIONS — Increased mean levels of triglycerides and systolic blood pressure may help to explain the higher prevalence of PAD in diabetic subjects compared with that in normal glucose tolerance subjects.

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The prevalence of cardiovascular disease has been shown to be higher in the diabetic (both type 1 and 2) population than in the nondiabetic population (1–3). Most research, however, has focused on coronary heart disease in diabetic subjects (4–7), and there are relatively few general population studies that have examined the prevalence of atherosclerotic peripheral arterial disease (PAD) according to diabetic status. Studies looking at intermittent claudication and a range of surrogate measures of PAD, including lower-limb amputations, pulse deficits, and medial calcification, have led to the general acceptance that PAD is more common in diabetic than in nondiabetic subjects (8–15).

The cause of this higher prevalence of PAD in diabetic subjects is unknown, but at least some of the effect may be related to differing levels of intermediate cardiovascular risk factors. For example, diabetic subjects have been found to have higher levels of hypertension (5,16–18), cigarette smoking (11,19,20), triglycerides (5,11,20,21), cholesterol (11,20,22), and other blood lipids (5,11,23) compared with nondiabetic subjects. Epidemiological studies have implicated many of these factors in the development of PAD in diabetes, but such studies have often relied on clinic-based samples of diabetic subjects and/or questionable measures of PAD (such as lower-limb amputations or pulse deficits). Information on the relationship between putative risk factors and PAD in diabetic subjects from the general population is, therefore, scarce (8).

In the present report, we examined data from the Edinburgh Artery Study, a prospective survey of 1,592 men and women selected from the general population. The aims of the study were to determine the risk factors for PAD in a diabetic population and to investigate whether raised levels of such risk factors in diabetic subjects might help to explain their increased risk of PAD.

RESEARCH DESIGN AND METHODS

Baseline study

The Edinburgh Artery Study began in 1988 as a cross-sectional survey of 809 men and 783 women aged 55–74 years. This population was selected at random, in 5-year age bands, from 11 general practices serving a range of socioeconomic and geographic areas throughout the city. The response rate was 65% (resulting in the final study pop-

From the Wolfson Unit for the Prevention of Peripheral Vascular Diseases, Department of Public Health Sciences, University of Edinburgh, Edinburgh, U.K.

Address correspondence and reprint requests to J.F. Price, Wolfson Unit for the Prevention of Peripheral Vascular Diseases, Department of Public Health Sciences, University of Edinburgh, Teviot Pl., Edinburgh, EH8 9AG, U.K. E-mail: jprice@srv1.med.ed.ac.uk.

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Abbreviations: ABPI, ankle-brachial pressure index; IGT, impaired glucose tolerance; OR, odds ratio; PAD, peripheral arterial disease; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.

ulation of 1,592), and follow-up of a sample of nonresponders showed no substantial bias. Subjects attended a university clinic to complete a questionnaire and have a comprehensive medical examination. Details of the study recruitment and examination process have been described (24). Ethics committee approval was given for this study, and informed consent was obtained from each subject.

Study measurements. Each subject completed a questionnaire that included the World Health Organization (WHO) intermittent claudication questionnaire (25) and a detailed section on smoking habit. After a 10-min rest in the supine position, systolic and diastolic (phase V) blood pressures were taken in the right arm using a Hawksley random zero sphygmomanometer. A single reading was taken unless the researcher experienced difficulty, in which case the reading was repeated until he or she felt confident with the reading. Ankle systolic blood pressures were taken in both legs using the random zero sphygmomanometer and a Sonicaid Doppler probe. Blood flow was detected where possible in the posterior tibial artery. The ankle-brachial pressure index (ABPI) was calculated as ankle divided by brachial systolic pressure, and the lesser ABPI from the two legs was used, since disease often occurs unilaterally. In the reactive hyperemia test that followed, ankle systolic pressure was measured 15 s after the release of a cuff occluding arterial flow just above the knee for 4 min at ~50 mmHg above systolic pressure. The timing was standardized using an electronic timer. This test was conducted to detect those subjects with peripheral arterial disease in whom the presence of medial arterial calcification (as may occur in diabetes) may have rendered measurement of the ABPI an unreliable marker of disease. Measuring the blood pressure 15–30 s after the cuff has been released has been shown to be the optimum time to distinguish between subjects with angiogram-positive disease and control subjects; in this situation, the test is >95% sensitive in detecting disease (26,27).

During the clinical examination, 20 ml of fasting blood was taken, after which subjects consumed 75 g glucose in the form of 335 ml of Solripe Gluctoza Health Drink (Strathmore Mineral Water, Forfar, Scotland, U.K.). A second blood specimen was taken 2 h after the oral glucose load. Standing height (without shoes) was measured to the nearest 5 mm using a free standing metal ruler on a heavy base. Weight without shoes and outer clothing was measured to the nearest 100 g on digital scales (Soehnle, Murrhardt, Germany). BMI as a measure of obesity was calculated as weight (in kilograms) divided by height (in meters) squared. In the laboratory, tests for serum total cholesterol, HDL cholesterol, triglycerides, thiocyanate, and plasma glucose were performed on a Cobas Bio analyzer (Roche, Welwyn Carden City, U.K.) using standard kits. LDL cholesterol was calculated using the formula: LDL cholesterol = total cholesterol − HDL cholesterol − triglycerides/5 (28). Quality control was measured by means of blind duplicate samples taken intermittently throughout the study.

Classification of PAD

For the purposes of this study, subjects were classified into two groups: *1*) the disease group, members of which had symptomatic PAD (determined by positive WHO intermittent claudication questionnaire) or major asymptomatic disease (ABPI ≤0.9 and drop in ankle systolic blood pressure during reactive hyperemia test of >20% or ABPI ≤0.7 or hyperemic drop of >35%), and *2*) the normal group, whose members met none of the above conditions and had ABPI >0.9 and hyperemic pressure reduction of <20%. This categorization has not been used in other studies, but results comparing the ABPI and reactive hyperemia test separately with angiography would suggest that it had adequate face validity; an ABPI of <0.9 has been shown to be up to 95% sensitive in detecting angiogram-positive disease (26). Also, we carried out duplex scanning on a subsample of cases confirming the presence of significant atherosclerotic disease. In the present study, 10% of subjects were classified as having PAD on the basis of an abnormal reactive hyperemia test alone (the remainder had intermittent claudication and/or an ABPI <0.9).

Diagnosis of diabetes/impaired glucose tolerance

Results of the oral glucose tolerance test were categorized according to WHO criteria (29). Subjects were classified as suffering from diabetes if *1*) they had been told by a doctor that they suffered from diabetes and were receiving treatment (insulin or oral therapy), *2*) the glucose concentration in the 2-h blood sample was ≥11.1 mmol/l, or *3*) because of a doctor diagnosis of diabetes, they did not undergo the oral glucose tolerance test (these subjects were classified a diabetic irrespective of whether or not the were on insulin or oral therapy). Impaired glucose tolerance (IGT) was diagnosed if th glucose concentration was between 7.8 and 11.1 mmol/l in the 2-h blood sample.

Data analysis

Data were analyzed on the Edinburgh University UNIX system using the SAS software package (version 6.11). Distributions of the fasted and 2-h glucose samples and triglycerides were positively skewed, thus logarithmic transformations were used in all analyses. The smoking history was sufficiently valid because reported consumption correlated well with mean thiocyanate level. Smoking status, defined as current ex-, or never smokers, was used as a categorical variable. Tests for differences in the age-adjusted mean levels of the risk factors between those with and without PAD were conducted using *t* tests for both the diabetic/IGT group and the normal glucose tolerance group. Logistic regression using the statistical package Proc GENMOD (30) was used to calculate the odds ratio (OR) for having PAD in a person who had diabetes/IGT compared with the risk of PAD for a person in the normal glucose tolerance group. Age and sex adjustments were made, and then each of the other potential related factors was individually included in a multivariate model.

RESULTS — There were 288 subjects (18.7%) who had diabetes (n = 91) or IGT (n = 197), compared with 1,253 subjects with normal glucose tolerance (51 subjects could not be classified because of missing data). Of the subjects with diabetes, 9% were insulin treated, 20% were on oral treatments, and 19% did not admit to taking any treatment other than diet modification; the remainder were previously undiagnosed diabetic patients who were detected using the oral glucose tolerance test. Figure 1 shows that PAD was more common in those with diabetes (22.4%, $P \leq 0.05$) and IGT (19.9%, $P \leq 0.05$), compared with those with normal glucose tolerance (12.5%). The prevalence of PAD was not significantly different ($P = 0.7$) in the diabetes and IGT groups, and these groups were combined to increase the power of the study. The OR for PAD among the diabetes/IGT group compared with the normal glucose tolerance group was 1.6 (95% CI 1.17–2.31, $P \leq 0.01$).

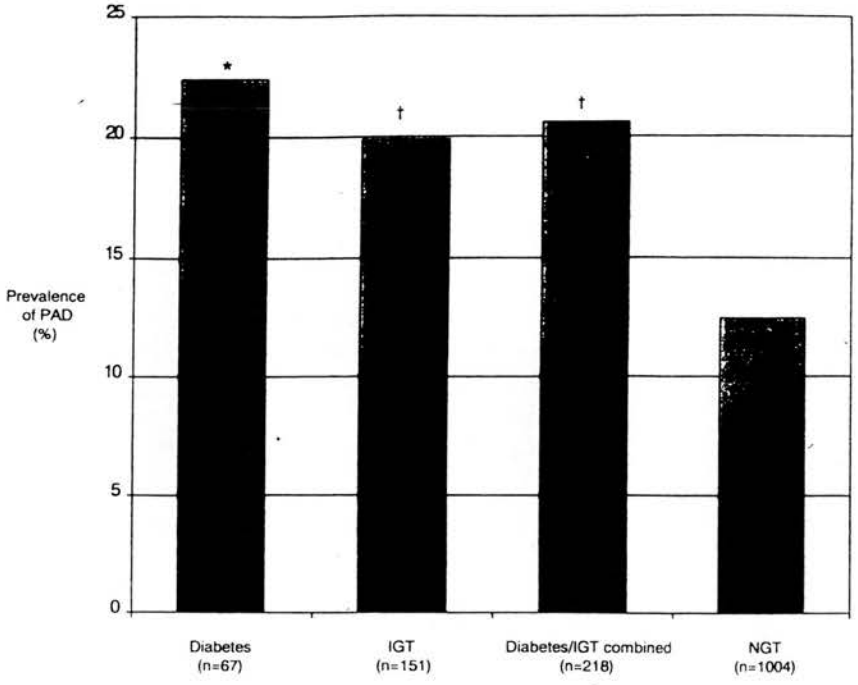

Figure 1—*Prevalence of PAD, categorized according to major asymptomatic disease (■) and intermittent claudication (■) in subjects with diabetes, IGT, and normal glucose tolerance (NGT). Difference in prevalence from NGT group: *P ≤ 0.05, †P ≤ 0.01.*

The mean levels of risk factors in subjects with diabetes/IGT with and without PAD are summarized in Table 1. Subjects with PAD were older than those without PAD ($P \leq 0.001$), so all risk factor levels were adjusted for age. Diabetic/IGT subjects with PAD had significantly higher mean levels of systolic blood pressure ($P \leq 0.01$) and plasma triglycerides ($P \leq 0.01$) compared with those without PAD. In addition, 74.1% of the diabetic group with PAD were current or ex-smokers, compared with only 48.1% of the diabetic subjects without PAD ($P \leq 0.05$). The mean levels of total and LDL cholesterol tended to be higher, and HDL cholesterol levels lower, in those with PAD compared with those without PAD, but these differences were not statistically significant ($P > 0.05$).

Table 2 shows that in subjects with normal glucose tolerance, those with PAD had significantly higher mean levels of systolic blood pressure, triglycerides, and total and LDL cholesterol ($P \leq 0.001$), and significantly lower levels of HDL cholesterol ($P \leq 0.05$), compared with those without PAD. Some 83.5% of the normal glucose tolerance group who had PAD were current or ex-smokers, compared with 61.6% of the normal glucose tolerance subjects without PAD ($P \leq 0.01$). There were no statistically significant differences in sex, BMI, or diastolic blood pressure.

Risk factor levels among the diabetes/IGT group with PAD and the normal glucose tolerance subjects with PAD were then compared (Tables 1 and 2, *column 1*). Patients in the diabetes/IGT group with PAD were older ($P \leq 0.05$), with higher mean levels of systolic blood pressure ($P \leq 0.01$) and plasma triglycerides ($P \leq 0.05$), than those with normal glucose tolerance and PAD. Differences in mean levels of the other risk factors between the two groups did not reach statistical significance, although the diabetes/IGT subjects with PAD had a more adverse risk factor profile in general than the normal glucose tolerance subjects with PAD.

The unadjusted OR for diabetes/IGT as a risk factor for PAD was 1.64 (95% CI 1.17–2.31, $P \leq 0.01$). Table 3 shows the ORs for diabetes/IGT as a risk factor for PAD compared with normal glucose tolerance, after adjusting for each of the common risk factors in turn. After adjusting for age and sex, diabetic subjects still had a higher risk of PAD (OR 1.45, 95% CI 1.03–2.04, $P \leq 0.05$). In multivariate analysis, those with diabetes/IGT no longer had a significantly higher risk of PAD after adjusting separately for systolic blood pressure (OR 1.22, 95% CI 0.85–1.73) and plasma triglycerides (OR 1.26, 95% CI 0.89–1.79). However, it should be noted that every risk factor, with the exception of smoking status, individually reduced the odds of diabetes as a risk factor for PAD. For example, after adjusting for age, sex, and HDL cholesterol, diabetic subjects still had higher levels of PAD, but it was of borderline significance (OR 1.37, 95% CI 0.97–1.94, $P = 0.08$). Simultaneous adjustment for both systolic blood pressure and triglycerides reduced the risk of PAD among diabetic patients to 1.11 (95% CI 0.78–1.58, $P > 0.05$).

CONCLUSIONS — In this population-based study, we have confirmed that the prevalence of PAD in subjects with diabetes or IGT is considerably greater than that in those with normal glucose tolerance.

Table 1—*Age-adjusted levels of risk factors in subjects with diabetes/IGT with and without PAD*

Risk factor	Diabetes/IGT		P value
	PAD	No PAD	
n	45	173	—
Age (years)	68.8 ± 0.8	65.1 ± 0.4	≤0.001
Sex (% M)	43.1	58.4	NS
Smoking status (% current or former)	74.1	48.1	≤0.05
Systolic blood pressure (mmHg)	161.8 ± 3.7	150.4 ± 1.8	≤0.01
Diastolic blood pressure (mmHg)	80.5 ± 1.9	78.9 ± 0.9	NS
BMI (kg/m²)	27.0 ± 0.7	26.8 ± 0.3	NS
Total cholesterol (mmol/l)	7.35 ± 0.22	6.99 ± 0.11	NS
LDL cholesterol (mmol/l)	5.61 ± 0.20	5.25 ± 0.10	NS
HDL cholesterol (mmol/l)	1.29 ± 0.06	1.38 ± 0.03	NS
Triglyceride (ln mmol/l)	0.69 ± 0.08	0.46 ± 0.04	≤0.01

Data are means ± SEM.

Table 2—*Age-adjusted levels of risk factors in normal glucose tolerant subjects with and without PAD*

Risk factor	Normal glucose tolerance		
	PAD	No PAD	*P* value
n	126	878	—
Age (years)	66.9 ± 0.5*	63.9 ± 0.2	≤0.001
Sex (% M)	50.4	52.7	NS
Smoking status (% current or former)	83.5	61.6	≤0.01
Systolic blood pressure (mmHg)	147.8 ± 1.9†	138.7 ± 0.7	≤0.001
Diastolic blood pressure (mmHg)	77.5 ± 1.1	76.1 ± 0.4	NS
BMI (kg/m²)	25.2 ± 0.3	25.0 ± 0.1	NS
Total cholesterol (mmol/l)	7.32 ± 0.12	6.89 ± 0.04	≤0.001
LDL cholesterol (mmol/l)	5.62 ± 0.11	5.14 ± 0.04	≤0.001
HDL cholesterol (mmol/l)	1.38 ± 0.04	1.47 ± 0.01	≤0.05
Triglyceride (ln mmol/l)	0.46 ± 0.04*	0.27 ± 0.02	≤0.001

Data are means ± SEM. Mean levels higher in diabetic/PAD than normal glucose tolerance/PAD group: *$P \leq 0.05$, †$P \leq 0.01$.

In our sample of Scottish men and women aged 55–74 years, 20.6% of those with diabetes/IGT had PAD, compared with 12.5% of those with normal glucose tolerance, supporting the commonly held view that diabetic patients are at increased risk of developing PAD (8). The present study included those with intermittent claudication and major asymptomatic PAD diagnosed using the WHO questionnaire, the ABPI, and the reactive hyperemia test. Because the WHO questionnaire has limited sensitivity (30) and use of the ABPI as a single measure of PAD can also be criticized (22,31), it is a major strength of this study that three measures of PAD were used. In addition, research has shown that subjects with asymptomatic PAD have an increased risk of cardiovascular complications, as well as claudicants (24,27), and asymptomatic PAD is more common in diabetic subjects (22,31).

The prevalence of PAD in diabetic subjects in previous studies varies widely as a result of different definitions of PAD and glucose tolerance, as well as different characteristics of populations surveyed (including age and race). In general, the prevalence of PAD among diabetic (22.4%) and IGT (19.9%) subjects reported in this study is higher than has been reported previously (11,15,18,22,32,33), probably because of the inclusion of subjects with major asymptomatic PAD. For example, Uusitupa et al. (11), using the WHO questionnaire, reported prevalences of intermittent claudication among newly diagnosed type 2 diabetic patents (45–64 years of age) of 9% in men and 3% in women. In a recent population-based study in England, the prevalence of PAD, defined by history, peripheral pulse deficits, and an ABPI of <0.9, was 8.7% in insulin-dependent subjects (mean age 39.3 years) and 23.5% in subjects with type 2 diabetes (mean age 67.7 years) (22). The authors concluded that peripheral vascular disease was significantly more common in the diabetic than in the nondiabetic subjects only when asymptomatic cases of PAD were added to both groups. The prevalence of PAD in the present study was almost identical to that found in a population-based study of 50- to 75-year-old Dutch Caucasians, which examined crural artery obstructions in diabetic and nondiabetic subjects using Doppler waveform analysis in addition to the ABPI (31).

Our findings of a worse cardiovascular risk factor profile in normal glucose tolerance subjects with PAD compared with those without PAD are consistent with previous studies (34). This included higher levels of cigarette smoking, systolic blood pressure, triglycerides, total and LDL cholesterol, and lower levels of HDL cholesterol. We also found that among the diabetes/IGT group a similarly adverse risk factor profile was present in those with PAD compared with those without PAD. This suggests that the same risk factors that are important in the development of PAD in normal glucose tolerance subjects are also important in diabetic subjects. However, in diabetic subjects, differences in several of the risk factors studied did not reach statistical significance, possibly because of the relatively small numbers of subjects affected.

The greatest difference in risk factor levels between diabetic subjects with and without PAD was found for systolic blood pressure, triglycerides, and cigarette smoking, suggesting that these risk factors may be particularly important in the development of PAD in diabetic populations. Hypertension and hypertriglyceridemia have previously been found to be risk factors in the development of PAD in diabetic subjects (5,11,16–18,21). Two studies found a relationship between hypertension and a low ABPI in diabetic subjects (16,17), while in the University Group Diabetes program, baseline serum triglyceride was significantly related to risk of intermittent claudication in men only (21). In Finland, a study using the WHO questionnaire as a single measure of PAD (11) found a relationship between triglycerides and PAD on univariate analysis. An association between both hypertension and hypertriglyceridemia and risk of intermit-

Table 3—*Odds of PAD according to diabetic status (diabetes/IGT vs. normal glucose tolerance) before and after adjustment for each risk factor*

Risk factors adjusted for	OR	95% CI	*P* value
Age and sex	1.45	1.03–2.04	≤0.05
Smoking status	1.65	1.16–2.34	≤0.01
Systolic blood pressure (mmHg)	1.22	0.85–1.73	0.3
Diastolic blood pressure (mmHg)	1.42	1.01–2.01	≤0.05
BMI (kg/m²)	1.42	1.00–2.01	≤0.05
Total cholesterol (mmol/l)	1.42	1.01–2.01	≤0.05
LDL cholesterol (mmol/l)	1.43	1.01–2.02	≤0.05
HDL cholesterol (mmol/l)	1.37	0.97–1.94	0.08
Triglyceride (ln mmol/l)	1.26	0.89–1.79	0.2
Systolic blood pressure and triglyceride*	1.11	0.78–1.58	0.6

Smoking status (current, ex, never) was used as a categorical variable. *This risk factor involves simultaneous adjustment for systolic blood pressure and triglycerides (log).

tent claudication was reported by the Framingham Study, which used its own questionnaire to ascertain claudication (5). In contrast, some other studies have failed to show a statistically significant relationship between mean levels of triglycerides and/or blood pressure and PAD in diabetic populations (19,21,22,33). The relationship between cigarette smoking and PAD in diabetic subjects has also been shown previously. A population-based study found an independent association between smoking and PAD upon multivariate analysis when both diabetic and nondiabetic subjects were pooled together (11). In a clinic-based study, logistic regression revealed a relationship between PAD and smoking in a sample of type 2 diabetic subjects (19).

Other studies have shown associations between cholesterol (total, HDL, and LDL) (5,11,22,23) and obesity (5,21) and manifestations of PAD among diabetic subjects. In our study, differences in total, LDL, and HDL cholesterol in diabetic/IGT subjects with PAD compared with those without PAD did not reach statistical significance, again possibly because of relatively small subject numbers.

Mean levels of several of the identified PAD risk factors were, in general, present at a more detrimental level in diabetic subjects than in nondiabetic subjects. For example, in subjects with PAD, systolic blood pressure and plasma triglycerides were both significantly higher in the diabetic subjects. These risk factors have previously been implicated in the development of PAD in diabetic subjects (5,11,16–18,21). A larger percentage of the nondiabetic PAD group, however, were current or ex-smokers (84.8%) compared with the diabetic PAD group (74.5%). The Framingham Study also found increased levels of cigarette smoking in male nondiabetic subjects compared with those in the male diabetic group, although women with diabetes tended to smoke more than women without diabetes (5).

It is possible that some of these differences in intermediary risk factors may contribute to the increased prevalence of PAD found in diabetic populations. To investigate this further, we calculated the risk of diabetes and then adjusted this for the other risk factors.

Adjustment for each risk factor examined in the present study reduced the impact of diabetic status on the likelihood of having PAD apart from cigarette smoking. This was due to the high percentage of never smokers in the diabetic subjects with PAD (25.9%) compared with the nondiabetic subjects with PAD (16.5%). Diabetic status was no longer a significant risk factor for PAD when adjustment was made for systolic blood pressure and triglycerides, suggesting that these two factors might be particularly important in helping to explain the increased prevalence of PAD in subjects with diabetes/IGT. The Framingham Study examined the influence of diabetes on manifestations of cardiovascular disease and did show a substantial reduction in the relative risk of PAD after adjusting for risk factors including cholesterol, smoking, and systolic blood pressure on multivariate analysis, compared with the age-adjusted figures. However, in this latter study the influence of diabetes on PAD remained significant after multivariate adjustment (5).

In conclusion, the results from this population-based study confirm that subjects with diabetes and IGT have a higher prevalence of PAD than those with normal glucose tolerance. Higher mean levels of triglycerides and systolic blood pressure in diabetic subjects may help to explain this higher prevalence. If the association between raised triglycerides and systolic blood pressure and the development of peripheral arterial disease in diabetic patients is found to be causal in future prospective studies, better control of these risk factors in diabetic patients should lead to a reduced incidence of PAD. Alternatively, it may be that some underlying phenomenon, such as insulin resistance syndrome (35), is responsible for both the elevation in systolic blood pressure and triglycerides and the higher prevalence of peripheral vascular disease. Indeed, we have shown previously that hyperinsulinemia is a risk factor for PAD, independent of blood pressure and serum lipids (36). Further longitudinal studies, including measurement of blood pressure, triglycerides, and insulin resistance, will be required to resolve these questions.

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Hyperinsulinaemia: a risk factor for peripheral arterial disease in the non-diabetic general population

Jacqueline F. Price, Amanda J. Lee and F. Gerald R. Fowkes

Background Peripheral arterial disease is a common complication of diabetes mellitus, and hyperinsulinaemia has been associated with an increased incidence of intermittent claudication in diabetic subjects. Our aim was to investigate the relationship between hyperinsulinaemia and peripheral arterial disease in the non-diabetic general population.

Methods Eighty-three cases with peripheral arterial disease and 88 age- and sex-matched controls were selected from non-diabetic participants in the Edinburgh Artery Study, a survey of 1592 men and women aged 55–74 years randomly selected from the general population.

Results Mean plasma insulin, 1 h after a 75 g oral glucose load, was higher in cases than in controls (73.6 versus 59.8 mU/l; $P < 0.05$). The relationship between insulin and disease was independent of blood pressure [odds ratio (OR) 2.04; 95% CI 1.11–3.74; $P \leq 0.05$] and partially independent of low- and high-density lipoprotein cholesterol and triglycerides (OR 1.86; 95% CI 0.99–3.48; $P \leq 0.1$). Mean 1 h insulin was higher in current or ex-smokers than in those who had never smoked ($P \leq 0.05$) and when smoking was added to the multivariate model, the relationship between insulin and disease diminished (OR 1.64; 95% CI 0.83–3.23; $P > 0.1$).

Conclusions In the non-diabetic general population, peripheral arterial disease is associated with post-glucose hyperinsulinaemia, independently of blood pressure, lipoproteins and triglycerides. Some of this association may be mediated by a relationship between hyperinsulinaemia and smoking.

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From the Wolfson Unit for Prevention of Peripheral Vascular Diseases, Department of Public Health Sciences, University of Edinburgh, Edinburgh, UK.

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Requests for reprints to Dr J.F. Price, Wolfson Unit for Prevention of Peripheral Vascular Diseases, Department of Public Health Sciences, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK.

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Introduction

Atherosclerotic disease of the lower limbs is a frequent and debilitating complication of diabetes mellitus and intermittent claudication is three to six times more common in diabetic than in non-diabetic subjects [1]. In its early stages, non-insulin-dependent diabetes is associated with insulin resistance and compensatory hyperinsulinaemia, and individuals with insulin-dependent diabetes are frequently hyperinsulinaemic because of peripheral administration of high-dose exogenous insulin [2]. It has therefore been postulated that hyperinsulinaemia is involved in the aetiology of peripheral arterial disease.

Although hyperinsulinaemia has been associated with an increased risk of ischaemic heart disease in four prospective studies [3–6], the role of insulin in other forms of atherosclerotic disease has been less well investigated. In particular, the association between insulin and the development of peripheral arterial disease in the non-diabetic general population is unknown. Two small studies indicated that non-diabetic men with atherosclerotic disease of the lower limbs had higher plasma insulin levels than healthy controls [7,8], but the studies did not consider the influence of other cardiovascular risk factors. In this respect, hyperinsulinaemia is known to be associated with hypertriglyceridaemia, low levels of high-density lipoprotein (HDL) cholesterol, and hypertension [9]. Also, smoking might affect insulin levels [10]. However, the degree to which the association between hyperinsulinaemia and cardiovascular disease is dependent on these other risk factors is unclear.

Our aim was to determine whether hyperinsulinaemia was associated with an increased risk of peripheral arterial disease in non-diabetic men and women, selected at random from the general population, and the extent to which this association was independent of other cardiovascular risk factors.

Methods

Study population

All the subjects in this nested case–control study were selected from the Edinburgh Artery Study, a prospective survey of 809 men and 783 women aged 55–74 years, selected at random from the general population. Details of the study recruitment, baseline examination and follow-up procedure have been described [11,12]. Study participants have now been followed-up for 5 years for cardiovascular events using annual validated questionnaires on cardiovascular history, intermittent claudication and angina [13], and information from general practitioners, hospitals, and the

Information Services Division (ISD) of the Scottish Office Home and Health Department. All cardiovascular events were further investigated using hospital or general practitioner records. At the 5-year follow-up examination, subjects completed a self-administered questionnaire which included validated questions on smoking, cardiovascular events and the World Health Organisation (WHO) angina and intermittent claudication questionnaires [13]. Right brachial systolic and diastolic blood pressures were recorded after 5 min rest using a random zero sphygmomanometer. Right and left posterior tibial systolic pressures were recorded in the supine position after 5 min rest, using a Doppler probe (Sonicaid, Chichester, UK) and random zero sphygmomanometer. The ankle brachial pressure index (ABPI) was calculated for each limb by dividing the posterior tibial by the brachial pressure. A 12-lead ECG was taken and coded independently by two observers using the Minnesota code [14].

Cases were selected from participants attending the 5-year follow-up examination if they had either: (i) a history of intermittent claudication according to the WHO intermittent claudication questionnaire, plus an ABPI ≤ 0.9 in at least one limb; or (ii) asymptomatic peripheral arterial disease indicated by an ABPI ≤ 0.85 in at least one limb. Controls were selected from the remaining study population if they had no WHO history of intermittent claudication and an ABPI ≥ 1.0 in both legs, no WHO or doctor recall history of cardiovascular disease (including angina, myocardial infarction or stroke) and no evidence of myocardial infarction or ischaemia on ECG. The controls were matched to the cases by sex and 5-year age band. Subjects were excluded from both the case and control groups for any of the following reasons: (i) known diabetes or newly-diagnosed diabetes according to a fasting plasma glucose ≥ 7.8 mmol/l [15], (ii) women using postmenopausal hormone-replacement therapy (to comply with requirements for a parallel study), or (iii) use of drugs affecting carbohydrate metabolism.

Risk factor measurement

All selected cases and controls underwent a standard 75 g oral glucose tolerance test, performed in ambulatory conditions and without dietary preparation after a 12 h overnight fast and after providing their informed consent. Venous samples for plasma insulin and blood glucose determination were taken before the test (fasting) and at 60 min after glucose administration. Samples were centrifuged at −4°C within 15 min of collection and plasma was immediately frozen and stored at −40°C. Before analysis, samples were stored at −20°C. Samples for total serum cholesterol, HDL cholesterol and triglycerides were also taken in the fasting state.

In the laboratory, plasma insulin concentration was measured using a microparticle enzyme immunoassay on an IMx analyser (Abbott Laboratories Ltd, Maidenhead, UK). The assay measurement range was 1–300 mU/l, with a sensitivity of 1 mU/l. Intra- and inter-assay coefficients of variation at an insulin concentration of 8.3 mU/l were 4.0 and 4.5%, respectively. The IMx assay showed no cross-reactivity with pro-insulin (< 0.005%) and no detectable cross-reactivity with C-peptide or glucagon. Plasma glucose concentration was measured by a timed end-point enzymatic method employing hexokinase and glucose-6-phosphate dehydrogenase using a Beckman Synchron CX5 multichannel analyser (Beckman Instruments Ltd, High Wycombe, UK). The assay analytical range was 0.3–38.8 mmol/l, sensitivity 0.3 mmol/l and inter-assay coefficient of variation 0.8%. Tests for total cholesterol and triglycerides were performed on a E750C dry chemistry analyser (Ortho Clinical Diagnostics Ltd, Hemel Hempstead, UK). Coefficients of variation were 1.4–2.1% for total cholesterol and 1.6–1.9% for triglycerides. HDL cholesterol was measured on a Cobas Mira Plus analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK) following chemical precipitation of other cholesterol-containing lipoproteins (coefficient of variation 3.4–4.6%). Low-density-lipoprotein (LDL) cholesterol was calculated using the formula: LDL cholesterol = total cholesterol − HDL cholesterol − triglycerides/5 [16].

Statistical analysis

Mean levels of risk factors were compared in study cases and controls, and the significance of differences were assessed by Student's t-test, using the statistical package SPSS-X (SPSS Inc., Chicago, USA). For triglycerides, fasting insulin, 1 h insulin, fasting glucose, 1 h glucose and insulin resistance, the values were log transformed because of skewed distributions. Geometric means and transformed confidence intervals were given for these variables. Cigarette smoking was calculated in pack-years (years of smoking multiplied by the average number of packs smoked per day) with the value zero entered for lifelong non-smokers. Square root of pack-years was taken to normalise the skewed distribution. Multiple logistic regression was used to investigate the independence of the relationship between post-glucose (1 h) insulin and disease with respect to age, sex and other cardiovascular risk factors; it was also used to test the independence of the association between smoking and disease with respect to 1 h insulin. Odds ratios were estimated from the logistic regression coefficients obtained from the statistical package BMDP (BMDP Statistical Software Inc., Los Angeles, USA).

Geometric means and transformed confidence intervals of 1 h insulin were calculated for never-smokers and ever-smokers (i.e. current and ex-smokers). Multiple linear regression analysis was used to determine the relationship between smoking status (ever-smoker versus never-smoker) and 1 h insulin. The following independent variables had forced entry into the final model: presence (cc = 1) or absence (cc = 2) of peripheral arterial disease; sex (male = 1, female = 2); age (using approximate mean for study population); smoking (never-smokers = 0, ever-smokers = 1). The final regression equation, predicted 1 h insulin = antilog

(4.59 – 0.18cc – 0.10sex + 0.002age + 0.26smoking), was used to give the predicted insulin levels for male never-smokers and ever-smokers separately.

Results

Subject characteristics

Age, sex and disease characteristics of the 83 cases (40 men and 43 women) and 88 controls (41 men and 47 women) are shown in Table 1. As expected from the matching, age and sex were similar in cases and controls. Mean ABPI was substantially lower in cases (0.7 in the cases, 1.1 in the controls; $P \leq 0.001$), 31% of whom had a history of intermittent claudication. Some of the cases also had evidence of angina (24%), previous myocardial infarction (16%) or previous stroke (4%).

Univariate analysis of risk factors

Table 2 shows the mean risk factor levels in cases and controls. Cases smoked more than controls and had higher mean blood pressure and serum triglycerides ($P \leq 0.05$). Cases also had slightly lower HDL cholesterol ($P \leq 0.1$). There was no significant difference in mean fasting insulin or fasting glucose between cases and controls ($P > 0.1$). However, mean insulin and glucose, 1 h after the oral glucose load, were higher in cases than in controls (1 h insulin, 73.6 mU/l in cases, 59.8 mU/l in controls; $P \leq 0.05$; 1 h glucose, 8.9 mU/l in cases, 8.1 mU/l in controls; $P \leq 0.05$). When the total study population was split by sex, 1 h insulin was significantly higher in men than in women (men 72.8 mU/l, women 59.4 mU/l; $P \leq 0.05$). Male cases had slightly higher 1 h insulin than male controls (male case 81.7 mU/l, male control 65.0 mU/l; $P \leq 0.1$), but the difference between female cases and controls did not quite reach the 10% significance level (female case 66.6 mU/l, female control 55.6 mU/l; $P = 0.11$).

Multivariate analysis of risk factors

The results of multiple logistic regression, used to examine the association between 1 h post-glucose insulin concentration and risk of disease, before and after adjustment for the other cardiovascular risk factors, are shown in Table 3. Higher 1 h insulin levels were associated with a significant increase in the risk of peripheral arterial disease [Odds ratio (OR) of disease for a one log unit increase in 1 h insulin concentration, adjusted for age and sex was 1.96; 95% CI 1.11–3.44; $P \leq 0.05$]. The strength of the association between disease and 1 h insulin was not significantly different between men (OR 2.24; 95% CI 1.00–5.06) and women (OR 1.82; 95% CI 0.78–4.30; $P > 0.1$ for difference between OR). Adjustment for the effects of systolic and diastolic blood pressure, with the sexes combined, did not greatly alter the association between disease and 1 h insulin concentration. Further adjustment for the effects of plasma LDL cholesterol, HDL cholesterol and triglycerides slightly weakened the relationship, but the odds ratio of 1.86 remained significant at the 10% level. However, when the effect of smoking was added to the model, the OR fell to 1.64 and was no longer significant.

Table 1 **Age, sex and cardiovascular disease characteristics of cases of peripheral arterial disease and controls**

	Cases (n = 83)	Controls (n = 88)
Age, mean years (SEM)	71.6 (0.6)	70.9 (0.5)
Males	48 (40)	47 (41)
Intermittent claudication	31 (26)	0
Mean ABPI (SEM)	0.7 (0.02)	1.1 (0.01)
Angina	24 (20)	0
Myocardial infarction	17 (14)	0
Stroke	4 (3)	0

ABPI, ankle–brachial pressure index. $P \leq 0.001$ for difference in ABPI between cases and controls. Values are expressed as percentage (number) unless otherwise stated.

Table 2 **Mean cardiovascular risk factor levels in cases of peripheral arterial disease and controls**

Risk factor	Cases (n = 83)	Controls (n = 88)	*P*
Systolic BP (mmHg)	154.8 (149.3–160.3)	143.6 (138.7–148.5)	≤ 0.01
Diastolic BP (mmHg)	83.6 (81.1–86.2)	80.6 (78.3–82.9)	≤ 0.1
Smoking (√packyears)	3.08 (2.38–3.78)	1.81 (1.27–2.34)	≤ 0.01
LDL cholesterol (mmol/l)	4.58 (4.36–4.80)	4.63 (4.42–4.84)	NS
HDL cholesterol (mmol/l)	1.20 (1.12–1.27)	1.29 (1.22–1.36)	≤ 0.1
Triglycerides (mmol/l)*	1.52 (1.39–1.67)	1.34 (1.23–1.45)	≤ 0.05
Fasting insulin (mU/l)*	6.4 (5.8–7.1)	6.1 (5.5–6.7)	NS
1 h insulin (mU/l)*	73.6 (65.0–83.3)	59.8 (53.2–67.3)	≤ 0.05
Fasting glucose (mmol/l)*	5.5 (5.4–5.7)	5.6 (5.5–5.7)	NS
1 h glucose (mmol/l)*	8.9 (8.4–9.6)	8.1 (7.6–8.6)	≤ 0.05

*Geometric mean of logged variable and transformed CI. BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NS, not significant. Values are expressed as means (95% CI).

Table 3 **Multivariate logistic regression of 1 h plasma insulin levels on peripheral arterial disease (all odds ratios adjusted for age and sex)**

Risk factors in model*	Odds ratio (95% CI)**	*P*
	1.96 (1.11–3.44)	≤ 0.05
BP	2.04 (1.11–3.74)	≤ 0.05
BP, LDL, HDL, Trig	1.86 (0.99–3.48)	≤ 0.1
BP, LDL, HDL, Trig, Cigs	1.64 (0.83–3.23)	0.17

*In addition to age and sex. **Odds ratio for a one unit increase on a log scale in 1 h post-glucose insulin. BP, systolic and diastolic blood pressure; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; Trig, triglycerides; Cigs, cigarette smoking (packyears).

One-hour glucose and insulin levels were highly correlated (Spearman's rank correlation coefficient; cases 0.34, $P \leq 0.05$; controls 0.49, $P \leq 0.001$). However, addition of 1 h glucose to the multivariate models in Table 3 made very little difference to the OR for 1 h insulin and disease, with 1 h insulin remaining associated with disease at the 10% level or less before adjustment for smoking (data not shown). Conversely, 1 h glucose was not associated with an increased risk of peripheral arterial disease after adjustment for age, sex and 1 h insulin (OR 1.60; 95% CI 0.52–4.95; $P > 0.1$).

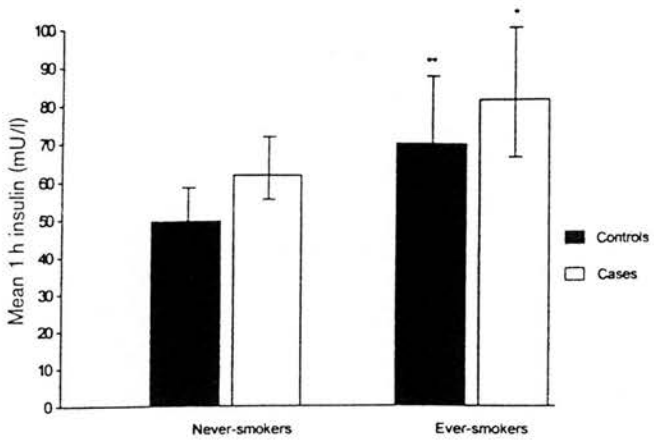
Insulin, smoking and disease

To investigate the possible inter-relationship between insulin levels and smoking, mean 1 h insulin was compared between ever- and never-smokers in both cases and controls (Fig. 1). Mean 1 h insulin was significantly higher in ever-smokers than in never-smokers in both groups (cases $P \leq 0.05$; controls $P \leq 0.01$). Multivariate linear regression analysis was performed with smoking as the independent variable and 1 h insulin as the dependent

variable. There was a significant association between smoking (ever- versus never-smoker) and 1 h insulin after adjustment for age, sex and peripheral arterial disease (B coefficient 0.26, SE 0.09; $P \leq 0.01$). This was equivalent to a predicted increase of 16.2 mU/l of 1 h insulin for ever-smokers compared with never-smokers, in men aged 70 years with peripheral arterial disease. The association between smoking and 1 h insulin did not alter greatly after additional adjustment for the other cardiovascular risk factors, including systolic and diastolic blood pressure, LDL and HDL cholesterol and triglycerides (B coefficient 0.22, SE 0.09; $P \leq 0.05$).

Despite the association between smoking and insulin, the relationship between peripheral arterial disease and smoking (age- and sex-adjusted OR of disease for a 1 √pack-year increase in smoking 1.19; 95% CI 1.05–1.35; $P \leq 0.01$) was only very slightly weakened after adjustment for 1 h insulin levels (OR 1.16; 95% CI 1.02–1.32; $P \leq 0.05$). Further adjustment for HDL cholesterol and triglyceride levels, which were correlated with smoking habit in subjects with peripheral arterial disease (Spearman's rank correlation coefficients; HDL –0.32, $P \leq 0.05$, triglycerides 0.24, $P \leq 0.05$) also had little effect on the association between smoking and disease (OR 1.15; 95% CI 1.01–1.31; $P \leq 0.05$).

Fig. 1



Geometric mean (transformed 95% CI) of 1 h insulin levels in never-smokers and ever-smokers, according to disease status. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ (ever-smoker versus never-smoker).

Discussion

Hyperinsulinaemia and peripheral arterial disease

Post-glucose insulin levels (but not fasting levels) were higher in non-diabetic subjects with symptomatic or severe asymptomatic peripheral arterial disease compared with healthy controls. This confirms the findings of earlier studies involving relatively small numbers of non-diabetic men with intermittent claudication [7,8]. However, unlike the present study, these studies did not take into account other, potentially confounding, cardiovascular risk factors. Raised blood pressure, triglycerides, LDL cholesterol and reduced HDL cholesterol are found to cluster in subjects with hyperinsulinaemia (syndrome X), and may explain at least some of the effects of insulin on disease [9]. Our study shows, for the first time, that the association between insulin and peripheral arterial disease is independent of blood pressure and only partially dependent on serum triglycerides, LDL cholesterol and HDL cholesterol. The association was also independent of blood glucose. The same independent relationship has been observed recently between insulin and coronary artery disease [6]. In addition, hyperinsulinaemia has been found to be related to the incidence of intermittent claudication in diabetic subjects after similar multivariate adjustment [17].

Our results are consistent with the hypothesis that hyperinsulinaemia is involved in the aetiology of peripheral arterial disease and may contribute to the higher incidence of disease in diabetic than in non-diabetic subjects. However, as with any cross-sectional or case–control study, the possibility that hyperinsulinaemia was a secondary manifestation of existing vascular disease must be considered [18]. Generalised atherosclerosis and reduced vasodilatory capacity in subjects with peripheral arterial disease may be associated with reduced glucose uptake into skeletal muscle and compensatory hyperinsulinaemia [19,20]. In addition, endothelial dysfunction caused by atherosclerosis might affect transport of insulin across capillary membranes [18], leading to elevated postglucose insulin levels in the presence of normal fasting levels, as in the present study. A prospective cohort study will be required to determine whether hyperinsulinaemia precedes the development of peripheral arterial disease.

An advantage of this study over numerous previous studies is that the insulin immunoassay did not cross-react with pro-insulin, confirming that the active insulin metabolite is associated with disease. The pathophysiological mechanism by which insulin might promote the development of atherosclerosis is unknown. In addition to its association with blood pressure, dyslipidaemia and possibly other cardiovascular risk factor such as plasminogen activator inhibitor-1 [2], insulin might have a direct pro-atherogenic effect on blood vessel walls [9]. Alternatively, hyperinsulinaemia might act as a marker for insulin resistance, with the latter determining cardiovascular risk [21].

Insulin, disease and sex

Another advantage of the present study is the inclusion of both men and women. There has been some debate concerning whether insulin is a risk factor for coronary artery disease in women as well as in men, as most large-scale prospective studies have been confined to men [3–6,22]. This question is particularly interesting since diabetes is a two- to three-fold stronger risk factor for coronary artery disease in women than in men [23], and the same appears to be true for peripheral arterial disease [17,24]. Mean post-glucose insulin levels were higher in both male and female cases than in sex-matched controls, although the

difference in women did not quite reach statistical significance. Furthermore, there was no significant difference in the strength of the association between insulin and disease in men compared with women. The most likely explanation of these results is that post-glucose insulin is a risk factor for peripheral arterial disease in both sexes, and that lack of a significant difference in insulin levels between female cases and controls reflected a small sample size.

Smoking, insulin and disease

We also demonstrated an association between smoking (a particularly strong risk factor for peripheral arterial disease [25]) and post-glucose insulin levels. Furthermore, this association explained at least some of the effects of insulin on disease. Smoking has not traditionally been associated with hyperinsulinaemia or syndrome X, although a recent report found higher insulin levels and greater insulin resistance associated with hypertriglyceridaemia and reduced HDL cholesterol levels in 20 smokers than in 20 non-smokers [10]. Fasting insulin levels were also raised in smokers and ex-smokers compared with non-smokers in 616 non-diabetic men from the general population [26]. This relationship was independent of other factors potentially affecting insulin sensitivity. It has been hypothesised that insulin resistance, hyperinsulinaemia and dyslipidaemia in cigarette smokers might be one mechanism by which smoking increases the risk of atherosclerotic disease [10]. Although we have confirmed a relationship between smoking and insulin levels and have further shown that this relationship is independent of other cardiovascular risk factors, neither insulin levels alone, nor in combination with HDL cholesterol and triglyceride levels, accounted for the effect of smoking on disease.

Conclusion

Raised post-glucose insulin levels were associated with atherosclerotic disease of the lower limbs in non-diabetic men and women from the general population. This association was at least partially independent of variations in blood pressure, lipoproteins and triglycerides and is consistent with the hypothesis that hyperinsulinaemia is involved in the aetiology of peripheral arterial disease in non-diabetic subjects. A positive correlation between smoking and raised insulin levels explained at least some of the relationship between insulin and peripheral arterial disease and merits further investigation.

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ELSEVIER

Steroid sex hormones and peripheral arterial disease in the Edinburgh Artery Study

J. F. Price, A. J. Lee, and F. G. R. Fowkes

Wolfson Unit for Prevention of Peripheral Vascular Diseases, Department of Public Health Sciences, University of Edinburgh, Edinburgh, UK

Although treatment with high dose exogenous sex hormones affects cardiovascular risk, the role of physiological levels of endogenous sex hormones in the development of atherosclerotic disease in men and women is unknown. Forty men and 43 women with peripheral arterial disease and 88 age- and sex-matched controls were selected from participants in the Edinburgh Artery Study, a random survey of 1592 men and women ages 55–74 years from the general population. Compared with sex-matched controls, male cases had higher systolic blood pressure (155.5 mmHg vs. 138.7 mmHg; $p \leq 0.01$) and waist hip ratio (0.92 vs. 0.89; $p \leq 0.05$) and female cases had higher lifetime smoking ($\surd$ packyears 2.14 vs. 1.03; $p \leq 0.05$). Mean estrone levels were slightly higher in male cases than controls (101.9 pmol/Liter vs. 92.1 pmol/Liter; $p = 0.09$), but this association lost significance after multivariate adjustment for age and body mass index. Mean levels of total and free testosterone, estradiol, and sex hormone binding globulin were not significantly different in cases compared with controls in either sex ($p > 0.1$). These results, in accordance with previous prospective studies on coronary artery disease, do not support a role for physiological levels of endogenous sex hormones in the development of peripheral arterial disease in men or postmenopausal women. (*Steroids* **62**:789–794, 1997) © *1997 by Elsevier Science Inc.*

Keywords: peripheral arterial disease; atherosclerosis; steroid sex hormones; estrogen; testosterone

Introduction

It is widely believed that the different hormonal milieu of men and women may help to explain the higher incidence of coronary artery disease in males than in females. Indeed, a protective role for estrogens in the development of cardiovascular disease has been suggested by the increased risk of both coronary artery disease[1] and atherosclerosis[2] in women following surgical menopause and by the lower risk of cardiovascular disease in postmenopausal women taking exogenous estrogen.[3] Moreover, both male and female sex hormone levels have been correlated with a variety of cardiovascular risk factors, including high density lipoprotein and low density lipoprotein cholesterol.[4] However, little is known about the relationship between endogenous sex hormones and risk of atherosclerosis. Most previous studies, confined to subjects with coronary artery disease, have measured only a limited range of sex hormones (often on frozen blood samples after prolonged storage with possible associated deterioration in hormone levels), neglected the possible importance of sex hormone binding globulin (SHBG) and the associated bioavailability of testosterone, or were confined to hospital populations.[5]

The aim of the present study was to explore the relationship between total testosterone, free testosterone, SHBG, estrone and estradiol, and atherosclerosis by comparing levels of these hormones in men and women with and without atherosclerotic disease of the lower limbs, selected at random from the general population.

Methods

All the subjects in this nested case-control study were selected from the Edinburgh Artery Study, a prospective survey of 809 men and 783 women ages 55–74 years, selected at random from the general population. Details of the study recruitment, baseline examination, and follow-up procedure have been described.[6,7] Study participants have now been followed up for 5 years for cardiovascular events using annual validated questionnaires on cardiovascular history, intermittent claudication[8] and angina,[8] and information from general practitioners, hospitals, and the Information Services Division of the Scottish Office Home and Health Department. All cardiovascular events were further investigated using hospital or general practitioner records.

At the 5-year follow-up examination, subjects completed a self-administered questionnaire that included validated

Address reprint requests to Dr. J. F. Price, Wolfson Unit for Prevention of Peripheral Vascular Diseases, Department of Public Health Sciences, University of Edinburgh, Edinburgh, EH8 9AG, UK.
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questions on smoking, cardiovascular events, and the WHO angina and intermittent claudication questionnaires.[8] Brachial systolic and diastolic blood pressures were recorded after 5 minutes rest using a random zero sphygmomanometer. Right and left posterior tibial systolic pressures were recorded in the supine position after 5 minutes rest, using a Doppler probe (Sonicaid, Chichester, UK) and random zero sphygmomanometer. The ankle brachial pressure index (ABPI) was calculated for each limb by dividing the posterior tibial by the brachial pressure. A 12-lead ECG was taken and coded independently by two observers using the Minnesota code.[9] Standing height (without shoes) was measured to the nearest 5 mm using a freestanding metal ruler and weight (without shoes and outer garments) was measured to the nearest 100 g on digital scales.

Cases were selected from participants attending the 5-year follow-up examination if they had either (a) a history of intermittent claudication according to the WHO intermittent claudication questionnaire, plus an ABPI ≤ 0.9 in at least one limb, or (b) asymptomatic peripheral arterial disease indicated by an ABPI ≤ 0.85 in at least one limb. An ABPI of less than 0.9 is known to be up to 95% sensitive and approximately 100% specific in detecting angiogram positive disease[10] and it is related to the severity of disease.[11] Controls were selected from the healthy study population if they had no history of intermittent claudication and an ABPI ≥ 1.0 in both legs, no history of cardiovascular disease (including angina, myocardial infarction or stroke), and no evidence of myocardial infarction or ischaemia on ECG. The controls were matched to the cases by sex and 5-year age band. Subjects were excluded from both the case and control groups for the following reasons: (a) known diabetes or newly diagnosed diabetes according to a fasting plasma glucose ≥ 7.8 mmol/Liter or the use of drugs affecting carbohydrate metabolism (to comply with requirements for a parallel study on insulin resistance) and (b) women with present or prior use of postmenopausal hormone replacement therapy.

Morning fasting venous blood samples were taken from all selected cases and controls for determination of total plasma testosterone, SHBG, estrone, and estradiol. Samples were centrifuged at $-4°C$ within 15 minutes of collection, and plasma was immediately frozen and stored at $-20°C$ for a maximum of six months before assay. Samples for total serum cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were taken at the same time, also in the fasting state. Waist and hip circumferences were measured to the nearest centimeter with the subject standing and breathing normally. Hip measurements were made at the level of the iliac crest and all measurements were made by a single observer. The waist hip ratio was calculated as the simple ratio of waist to hip circumference.

In the laboratory (Reproductive Medicine Laboratory, University of Edinburgh), estradiol was measured using an in-house radioimmunoassay following ether extraction of serum. Estradiol-3-CME iodohistamine was used as tracer, and the antibody was sheep anti-estradiol (lot BW 26/9/80). Bound and free tracer were separated using dextran-coated charcoal. Estrone was measured using an in-house ELISA following ether extraction of serum. The primary antibody was rabbit anti-estrone-3-glucuronide-BSA (obtained from the MRC/AFRC Comparative Physiology Group, Institute of Zoology, London, UK) with estrone-3-glucuronide conjugated to horseradish peroxide as tracer and o-phenylenediamine dihydrochloride as substrate. SHBG and total testosterone were measured by radioimmunoassay using commercial kits (Orion Diagnostica, Espoo, Finland and Medgenix, Springfield, Missouri, USA, respectively). All samples were assayed in duplicate, and the sample was assayed again if the values for the individual duplicates differed by more than 5%. Inter- and intra-assay coefficients of variation were $\leq 9.2\%$ for estradiol, $\leq 9.9\%$ for estrone, $\leq 9.3\%$ for SHBG, and $\leq 11.7\%$ for total testosterone. For subjects whose hormone levels were found to be below the level of sensitivity of the total testosterone assay (0.18 nmol/Liter) and the estradiol assay (30 pmol/Liter), these values were used in the analysis. Free testosterone was calculated using the Free Androgen Index according to the equation free testosterone (pmol/Liter) = [1000 × total testosterone (nmol/Liter)]/sex hormone binding globulin (nmol/Liter).

Tests for total cholesterol and triglycerides were performed on a E750C dry chemistry analyzer (Ortho Clinical Diagnostics, Raritan, New Jersey, USA). Coefficients of variation were 1.4–2.1% for total cholesterol and 1.6–1.9% for triglycerides. HDL cholesterol was measured on a Cobas Mira Plus analyser (Roche Diagnostics Ltd) following chemical precipitation of other cholesterol-containing lipoproteins (coefficient of variation 3.4–4.6%). Low density lipoprotein (LDL) cholesterol was calculated according to the formula of Friedewald et al.[12]: LDL cholesterol = total cholesterol − HDL cholesterol − triglycerides/5.

Mean levels of risk factors were compared in study cases and controls, and the significance of differences were assessed by *t*-test, using the statistical package SPSS-X. For triglycerides, SHBG, and estrone, the levels were log transformed because of skewed distributions, and geometric means were given for these variables. Cigarette smoking was calculated in pack years (years of smoking multiplied by the average number of packs smoked per day) with the value zero entered for lifelong nonsmokers. Square root of pack years was taken to normalize the skewed distribution. Multiple logistic regression was used to investigate the relationship between sex hormones and disease after controlling for age, body mass index (BMI), and other cardiovascular risk factors. Odds ratios and 95% confidence intervals were estimated from the regression coefficients obtained from the statistical package BMDP.

Results

Age and disease characteristics of the male cases ($n = 40$) and controls ($n = 41$) and female cases ($n = 43$) and controls ($n = 47$) are shown in Table 1. As expected from the matching, age was similar in cases and controls. Mean ABPI was substantially lower in both male and female cases, over 30% of whom had a history of intermittent claudication. Several of the cases also had evidence of angina or previous myocardial infarction.

Table 2 shows the mean risk factor levels in male cases and controls. Male cases had significantly higher mean systolic blood pressure and greater waist to hip ratios than

Table 1 Age and Disease Characteristics of Male and Female Cases of Peripheral Arterial Disease and Controls

	Men		Women	
	Cases (n = 40)	Controls (n = 41)	Cases (n = 43)	Controls (n = 47)
Age, mean years (SE)	71.9 (0.8)	71.4 (0.82)	71.3 (0.8)	70.5 (0.7)
Intermittent claudication, % (n)	30 (12)	0	33 (14)	0
Mean ABPI (SE)	0.70 (0.02)	1.14 (0.02)	0.72 (0.02)	1.10 (0.01)
Angina, % (n)	28 (11)	0	21 (9)	0
Myocardial infarction, % (n)	20 (8)	0	14 (6)	0

ABPI, ankle brochial pressure index.

controls ($p \leq 0.05$). They also had slightly higher serum triglyceride levels, BMI, and lifetime smoking ($p \leq 0.1$). There was no significant difference in mean levels of total testosterone, SHBG, free testosterone, or estradiol between cases and controls ($p > 0.1$), but mean estrone was slightly higher in the cases (101.9 pmol/Liter vs. 92.1 pmol/l, $p = 0.09$).

Table 3 gives mean levels of the same risk factors in female cases and controls. Female cases had significantly higher lifetime smoking than controls ($p \leq 0.05$) and slightly lower HDL cholesterol ($p \leq 0.1$). There was no significant difference in mean levels of total testosterone, SHBG, free testosterone, estradiol, or estrone between cases and controls ($p > 0.1$).

The prevalence of thyroid disease, which can affect SHBG concentrations, was low (data not shown). Five female cases, two female controls, and no male subjects were taking thyroxine (which can increase SHBG levels) and no subjects of either sex were being treated for hypothyroidism. When female subjects taking thyroxine were excluded from the analysis, there remained no significant difference in SHBG levels between cases and controls (mean SHBG 44.6 cases; 48.6 controls; $p = 0.46$).

The results of multiple logistic regression, used to examine the association between steroid sex hormones and risk of disease after adjustment for the other cardiovascular risk factors, are shown in Table 4. None of the sex hormones was associated with an increased or decreased risk of peripheral arterial disease, including serum estrone in men, after multivariate adjustment for age and BMI. Further adjustment for systolic blood pressure, smoking, LDL and HDL cholesterol, and triglycerides had no major effect on these results.

Discussion

To our knowledge, this is the first report of a study investigating the relationship between endogenous sex hormones and peripheral arterial disease. We were able to measure a wide range of sex hormones, as well as SHBG, using plasma that had been stored for only a limited time, and subjects from the general population rather than hospitals. We found little evidence to support the suggestion that testosterone (total or free), SHBG, estradiol, or estrone were risk factors for peripheral arterial disease in either sex. The most important risk factors were systolic blood pressure, cigarette smoking, triglycerides, BMI and waist hip ratio in men, and smoking and reduced HDL cholesterol in women.

Men

Several case-control studies of endogenous hormones in men with coronary artery disease found higher estrogen

Table 2 Mean Risk Factor Levels in Male Cases of Peripheral Arterial Disease and Controls

	Male cases (n = 40)		Male controls (n = 41)		
	Mean	95% CI	Mean	95% CI	p-value
Systolic BP, mmHg	155.5	(146.5,164.4)	138.7	(132.0,145.4)	0.004
Smoking, \ packyears	4.12	(3.13,5.11)	2.85	(1.91,3.79)	0.07
Total cholesterol, mmol/Liter	6.23	(5.91,6.55)	6.21	(5.88,6.54)	0.94
LDL cholesterol, mmol/Liter	4.38	(4.07,4.68)	4.48	(4.17,4.79)	0.65
HDL cholesterol, mmol/Liter	1.07	(0.97,1.17)	1.12	(1.03,1.21)	0.47
Triglycerides, mmol/Liter*	1.53	(1.32,1.78)	1.28	(1.13,1.45)	0.07
BMI, kg/m^2	26.5	(25.4,27.6)	25.2	(24.3,26.0)	0.06
WHR	0.92	(0.90,0.94)	0.89	(0.87,0.91)	0.04
Total testosterone, nmol/Liter	13.6	(12.0,15.3)	14.9	(13.3,16.5)	0.28
SHBG, nmol/Liter*	34.6	(30.4,39.3)	39.5	(35.6,43.8)	0.12
Free testosterone, pmol/Liter	403.4	(354.6,452.2)	379.2	(336.4,422.0)	0.47
Estrone, pmol/Liter*	101.9	(94.8,109.5)	92.1	(84.2,100.8)	0.09
Estradiol, pmol/Liter	97.9	(88.6,107.3)	106.1	(97.3,114.9)	0.21

*geometric mean of logged variable.
BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; BMI, body mass index; WHR, waist hip ratio; SHBG, sex hormone binding globulin.

Table 3 Mean Risk Factor Levels in Female Cases of Peripheral Arterial Disease and Controls

	Female cases (n = 43)		Female controls (n = 47)		
	Mean	95% CI	Mean	95% CI	p-value
Systolic BP, mmHg	154.2	(147.4,161.0)	147.8	(141.0,154.7)	0.20
Smoking, $\sqrt{}$ packyears	2.14	(1.24,3.04)	1.03	(0.51,1.55)	0.04
Total cholesterol, mmol/Liter	6.81	(6.46,7.16)	6.88	(6.57,7.19)	0.77
LDL cholesterol, mmol/Liter	4.77	(4.45,5.08)	4.77	(4.49,5.05)	0.99
HDL cholesterol, mmol/Liter	1.31	(1.22,1.40)	1.44	(1.35,1.53)	0.06
Triglycerides, mmol/Liter*	1.50	(1.33,1.71)	1.39	(1.26,1.43)	0.34
BMI, kg/m^2	26.1	(24.9,27.4)	26.5	(25.4,27.6)	0.67
WHR	0.88	(0.85,0.90)	0.87	(0.85,0.90)	0.83
Total testosterone, nmol/Liter	0.62	(0.50,0.73)	0.72	(0.58,0.86)	0.29
SHBG, nmol/Liter*	45.0	(38.2,53.1)	49.0	(42.4,56.7)	0.45
Free testosterone, pmol/Liter	15.3	(11.24,19.35)	16.0	(12.4,19.5)	0.80
Estrone, pmol/Liter*	81.26	(73.96,89.27)	82.96	(77.92,88.33)	0.72
Estradiol, pmol/Liter	47.02	(42.76,51.29)	49.72	(45.44,54.00)	0.39

*geometric mean of logged variable.
BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; BMI, body mass index; WHR, waist hip ratio; SHBG, sex hormone binding globulin.

levels and lower testosterone levels in survivors of myocardial infarction compared with controls.[5] Although we found slightly higher levels of estrone in male cases than in male controls, this relationship was only weakly significant and became nonsignificant after adjustment for age and BMI. Since obesity is associated with altered sex hormone levels and is also a risk factor for cardiovascular disease, it is an important confounding factor. Neither mean estradiol nor total testosterone levels were significantly different in men with peripheral arterial disease compared with controls. These results are consistent with those of prospective studies, in which none of the three sex hormones, estradiol,[13–17] estrone,[13,16,17] or total testosterone[13–16] predicted subsequent myocardial infarction[13–15,17] or the development of symptomatic cardiovascular disease.[16] It is likely that the altered hormone levels found in myocardial infarction survivors were a consequence of myocardial infarction, rather than a precursor. In our population, only 20% of male cases and 14% of female cases had a history of myocardial infarction and none of these events occurred within the year before hormone measurement.

More than 90% of total testosterone circulates bound to proteins, predominantly SHBG, and it is possible that only the free (bioavailable) testosterone fraction is important in determining cardiovascular risk. However, neither in the present study, nor in prospective studies of coronary artery disease, was free testosterone associated with the presence or development of atherosclerotic disease.[13,16] More recently, it has been suggested that SHBG itself might be a risk factor for cardiovascular disease, largely because of observed correlations between SHBG and cardiovascular risk factors such as HDL cholesterol and insulin.[18] How-

Table 4 Multivariate Logistic Regression of Steroid Sex Hormones on Peripheral Arterial Disease

	Men			Women		
Hormone factors adjusted for	OR	95% CI	p-value	OR	95% CI	p-value
Total testosterone**						
age,BMI	0.84	(0.52,1.36)	0.48	0.79	(0.51,1.23)	0.30
age,BMI,sBP,cigs,LDL,HDL,TGs	0.75	(0.41,1.38)	0.37	0.70	(0.41,1.17)	0.19
SHBG*						
age,BMI	0.43	(0.12,1.55)	0.21	0.59	(0.23,1.49)	0.27
age,BMI,sBP,cigs,LDL,HDL,TGs	0.50	(0.10,2.60)	0.44	0.72	(0.24,2.15)	0.57
Free testosterone**						
age,BMI	1.21	(0.74,1.99)	0.46	1.98	(0.63,1.51)	0.92
age,BMI,sBP,cigs,LDL,HDL,TGs	1.10	(0.61,1.99)	0.77	0.77	(0.45,1.30)	0.35
Estrone*						
age,BMI	3.36	(0.55,20.6)	0.20	0.69	(0.14,3.49)	0.66
age,BMI,sBP,cigs,LDL,HDL,TGs	2.13	(0.21,21.2)	0.55	0.71	(0.12,4.43)	0.73
Estradiol**						
age,BMI	0.74	(0.46,1.19)	0.22	0.82	(0.53,1.28)	0.39
age,BMI,sBP,cigs,LDL,HDL,TGs	0.67	(0.36,1.26)	0.24	0.79	(0.47,1.32)	0.39

OR: *Odds ratio for a one unit increase on a log scale in steroid sex hormone.
**Odds ratio for a 1 SD increase in steroid sex hormone (total testosterone 5.22 mnol/Liter men, 0.45 nmol/Liter women; free testosterone 148 pmol/Liter men, 12.85 pmol/Liter women; estradiol 29.54 pmol/Liter men, 14.53 pmol/Liter women).

ever, we found no significant difference in the levels of SHBG between men with and without peripheral arterial disease, consistent with previous case-control[19,20] and prospective[16] studies on sex hormone binding concentration and cardiovascular disease in men.

Women

Postmenopausal women taking pharmacological doses of exogenous estrogens reduce their risk of cardiovascular disease,[3] However, we did not find any significant difference in endogenous estrone or estradiol levels between women with and without peripheral arterial disease, suggesting that physiological doses of estrogens may not be associated with disease. Correspondingly, no association has been found between circulating estrone levels and prevalent heart disease,[21] nor between estrone or estradiol and the development of symptomatic cardiovascular disease.[22]

Neither testosterone (total or free), nor SHBG levels differed between women with and without peripheral arterial disease in the present study. Previous information on endogenous testosterone levels and atherosclerotic disease in women is scarce. Although women with testosterone excess associated with chronic anovulation had an increased risk of myocardial infarction, in a single prospective study,[23] neither total nor free testosterone predicted fatal cardiovascular disease in postmenopausal women.[22] However, low SHBG levels did increase the 12-year incidence of cardiovascular disease in postmenopausal women.[24]

An important consideration in any negative study is whether the study had sufficient power to detect a significant difference. With our sample size and at a power of 80% ($\alpha = 0.05$), we could detect a difference in each sex hormone equivalent to approximately half a standard deviation of its sex-specific distribution (for example, a difference in total testosterone of 2.55 nmol/Liter in men and 0.27 nmol/Liter in women). It is possible that smaller differences might have been detected using larger subject numbers, but the physiological relevance of such small differences is questionable. It is also possible that our results could have been affected by the sensitivity of the hormone assays; in our laboratory, 18% and 22% of women were below the sensitivity range for estradiol and total testosterone, respectively, but no men were below the sensitivity range of any of the sex hormones and no women were below the sensitivity range for estrone or SHBG. Diurnal variation in hormone levels should not have affected the results because all samples were taken fasting at the same time in the morning.

The population studied in this investigation was relatively elderly (mean age 71 years). The fact that exogenous estrogens reduce cardiovascular risk in postmenopausal women suggests that exposure to sex hormones, even in later life, may be an important factor in determining disease. However, the latency period of cardiovascular disease is long, and it is also possible that any effects of endogenous sex hormones on disease are more important earlier in life, during the development phase of atherosclerosis. This is a particularly important consideration in women, because estrogen levels fall substantially during the menopause. Unfortunately, study of premenopausal women would be difficult due to their low risk of cardiovascular disease (necessitating prolonged follow-up) and difficulties in determining average hormone concentrations during the menstrual cycle.

In conclusion, this study does not support the hypothesis that endogenous sex hormone levels are important in determining risk of atherosclerotic disease in elderly men and postmenopausal women. The role of endogenous sex hormones in cardiovascular disease now needs to be addressed in younger populations, in particular to determine the significance of observed associations between sex hormones and cardiovascular risk factors

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